



Chemistry, Manufacturing and Controls (CMC) Review Memorandum

To: File of STN 125640/0
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Subject: Final review of Adventitious Agents Safety Information in Instituto Grifols, S.A.'s
original BLA for Fibrin Sealant (Human)
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1. Executive Summary

This memorandum summarizes the review of Adventitious Agents Safety Information in the original Biologics License Application (BLA) under STN 125640/0 and amendments submitted by Instituto Grifols, S.A. (IG) for Fibrin Sealant (Human). As described below, the measures taken by IG to control adventitious agents in the manufacture of Fibrin Sealant (Human) final drug product (FDP) are acceptable; together with the Post-Marketing Commitment (PMC) from IG, listed at the end of this memo, we recommend approval of the BLA under STN 125640/0.

2. Background

Fibrin Sealant (Human) is a frozen, sterile, two-component solutions obtained from human plasma pools. This product consists of Human Fibrinogen and Human Thrombin components. Fibrin Sealant (Human) is manufactured at IG's facility located at Barcelona, Spain. It is supplied as a single-use kit containing two pre-filled syringes, each with a sterile frozen solution, assembled on a syringe holder. This product is available in four pack sizes: 2 mL, 4 mL, 6 mL, or 10 mL (total volume) per kit.

3. Summary of Review

3.1. Flow diagrams of the manufacturing processes

Human Fibrinogen component

1. (b) (4) plasma
2. (b) (4) of Fraction I
3. (b) (4) of Fraction I
4. **Solvent/Detergent treatment** (0.3% Tri(n-butyl)phosphate (TnBP, v/v), and 1.0% Polysorbate 80 (v/v) at 25.5 – 28.5°C for 6 – 6.5 hours)
5. First glycine precipitation
6. Second glycine precipitation
7. Third glycine precipitation
8. (b) (4)
9. (b) (4) and **nanofiltration** ((b) (4) 35N and 20N nanofilters)
10. (b) (4) and final formulation
11. Bulk filtration and aseptic filling
12. Assembling the syringes of Fibrinogen for Fibrin Sealant (Human)
13. Sterilizing with (b) (4)
14. Final packaging and freezing
15. Drug product

Human Thrombin component

1. (b) (4) plasma
2. (b) (4) of Fraction I
3. (b) (4) of the prothrombin complex by (b) (4)
4. (b) (4)

5. **Solvent/Detergent treatment** (0.3% TnBP (v/v), and 1.0% Polysorbate 80 (v/v) at 24 – 26°C for 6 – 6.5 hours)
6. Purification by SP-Sepharose ion-exchange chromatography
7. (b) (4) and final concentration
8. (b) (4)
9. **Nanofiltration** (double (b) (4) 15N nanofilters)
10. Bulk filtration and aseptic filling
11. Assembling the syringes of Thrombin for Fibrin Sealant (Human)
12. Sterilizing with (b) (4)
13. Final packaging and freezing
14. Drug product

Product reviewer’s comment: There are two dedicated viral clearance steps in the manufacturing processes of Human Fibrinogen component or Human Thrombin component (bolded in the above flow diagrams) which are acceptable. Glycine precipitation in the manufacture of Human Fibrinogen component also contributes to viral clearance. Two additional steps (i.e., Fraction I precipitation and SP-Sepharose XL chromatography) in the manufacture of Human Thrombin component contribute to viral clearance as well. The validation reports of these viral clearance steps were reviewed, and the results demonstrate that these steps are capable of inactivating or removing viruses, thus lowering the potential of viral contamination. My review will focus separately on each of the two components of Fibrin Sealant (Human).

3.2. Evaluation of non-viral adventitious agents’ elimination capacity - Fibrin Sealant (Human)

IG manufactures the Fibrin Sealant (Human) FDP according to GMP regulations. For the non-viral adventitious agents, such as bacteria, fungi, and mycoplasma, the potential contamination of these agents is well controlled through the use of validated cleaning/sanitization procedures (such as the use of (b) (4) solutions), and in-process filtration steps, including (b) (4) sterile filtration. The final container of Fibrin Sealant (Human) is also guaranteed to be free of non-viral adventitious agents by the testing for Sterility and Endotoxins.

Product reviewer’s comment: The measures taken by IG to control non-viral adventitious agents in the manufacture of Fibrin Sealant (Human) are acceptable.

3.3. Evaluation of transmissible spongiform encephalopathy agents’ elimination capacity - Fibrin Sealant (Human)

- Human plasma

To minimize the risk of transmissible spongiform encephalopathy (TSE) agents, donors who are potentially at risk are excluded from plasma donation, as specified in the current FDA guidance regarding donations collected in the U.S (*Guidance for Industry Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products, May 2010*). For example, potential risk donors are individuals that spent three months or more cumulatively in the U.K. from the beginning of 1980 through the end of 1996.

- Human albumin

Human albumin is used in the formulation of the Human Thrombin component. This excipient is manufactured by IG, and is licensed in the U.S. under the trade name of Human Albumin Grifols® (STN 103352).

No other materials of animal or human origin are used in the manufacture of Fibrin Sealant (Human). No other excipients of human or animal origin are used in the formulation of Fibrin Sealant (Human) FDP.

Product reviewer’s comment: Based on the information above, the potential risk of contaminating TSE agents is low in Fibrin Sealant (Human).

3.4. Evaluation of viral elimination capacity - Fibrin Sealant (Human)

3.4.1. Selecting and testing the US sourced human plasma for the absence of detectable viruses

Only human plasma collected in centers licensed by FDA can be used for the manufacture of Fibrin Sealant (Human) for the U.S. market. A physical examination and suitable answers to an extensive questionnaire are required for all donors before each donation. Each donation is tested and found non-reactive for the presence of Hepatitis B surface Antigen, antibodies against Human Immunodeficiency Virus (HIV)-1/2 and Hepatitis C Virus (HCV). TSE was discussed in Section 3.3 of this review memo. Thus, donor selection is performed in accordance with the requirements of the 21 CFR and the respective FDA guidelines. Also, human plasma used for the manufacture of Fibrin Sealant (Human) is Source Plasma (21 CFR 640.60).

Product reviewer’s comment: IG did not provide the relevant information on nucleic acid technique (NAT) tests performed on human plasma pools that will be used for the production of Fibrin Sealant (Human). To better estimate the potential virus load in the starting material, we asked IG to submit the limit of detection (LOD) of each NAT test used for detection of HIV, Hepatitis B Virus (HBV), HCV, Hepatitis A Virus (HAV), or human parvovirus B19 (B19V) in the (b) (4) or mini-pool (b) (4), and the manufacturing plasma pool.

In Section 3.2.S.2.3. *Control of Materials* (Human Fibrinogen or Human Thrombin), IG stated that the (b) (4) and the manufacturing plasma pools have been tested, and found to be non-

reactive for viral markers of infection, including B19V. We asked IG to submit the acceptance limits for the level of B19V DNA in the (b) (4) and the manufacturing pool, and the results of all the plasma pools tested so far based on their quantitative NAT for B19V.

Based on these comments, an information request (IR) was sent to IG on April 18, 2017, and IG responded in Amendment #26 on 25 April, 2017. IG response is summarized as follows:

Human plasma used in the manufacture of Fibrin Sealant (Human) is tested by (b) (4) FDA-licensed testing facilities: (b) (4)

- 1) Mini-pool (b) (4): Each pool is tested for the absence of viral genome of HAV, (b) (4). Although the limits of detection are different among the different testing facilities, the highest values of 95% LOD for HAV, (b) (4).
For B19V, the highest value of 95% LOD is (b) (4).
- 2) Manufacturing plasma pool: Each pool is tested to be negative for (b) (4). These pools can be released only if they are also non-reactive for HBV, HCV, HIV-1, and have titer of B19V $\leq 10^4$ IU/mL. The value of 95% LOD of each NAT assay performed by IG is (b) (4).
- 3) IG tested (b) (4) manufacturing plasma pools after March 2014, and none of the B19V test results in the pools is $> 10^4$ IU/mL.

Product reviewer's comments: Based on the LOD data of each NAT assay, and the viral clearance capacities discussed in the following sections (with reference to viral reduction factors summarized in the tables on page 15 of this memo), the proposed commercial manufacturing process may result in relatively high safety margin (at least (b) (4) for HIV-1, HBV, HCV, and HAV). Additionally, IG confirmed that the limit of B19V detection in each of the manufacturing plasma pool ((b) (4) for Human Fibrinogen and (b) (4) for Human Thrombin) is $\leq 10^4$ IU/mL. The historical data also support this criterion, which is consistent with the requirement of the relevant FDA guidance (*Guidance for Industry Nucleic Acid Testing (NAT) to Reduce the Possible Risk of Human Parvovirus B19 Transmission by Plasma-Derived Products, July 2009*). Therefore, this response is acceptable.

3.4.2. Validation of viral clearance in selected steps of the manufacturing process of the Human Fibrinogen component

3.4.2.1. Down-scale validation – Human Fibrinogen component:

To support the relevant viral clearance studies, each down-scale system (i.e., S/D treatment, nanofiltration, and glycine precipitation) should be representative of the proposed commercial manufacturing. However, IG did not provide the down-scale factor relative to the proposed commercial-scale in each of these viral clearance studies. An IR, based on this comment, was

sent to IG on August 7, 2017, and IG responded in Amendment #43 on September 8, 2017. IG response is summarized as follows:

- 1) S/D treatment: IG provided the data on the qualification of the down-scale system used for viral inactivation by S/D treatment (*Study No. IG_ISVR 01-194_ING* and *IG_BRL-ISVR AD41JV.028925.BUK*). In these studies, the proposed commercial-scale process was scaled down in a ratio of (b) (4) (HIV, PRV, and BVDV) or (b) (4) (WNV). Additionally, the critical parameters including the (b) (4) were adjusted to the worst-case scenario. (b) (4)
- 2) Nanofiltration ((b) (4) 35N and (b) (4) 20N (b) (4)): IG provided the qualification of the down-scale system used for viral removal by nanofiltration (*Study No. IG_ISVR 03-142_ING*). In the study, the proposed commercial-scale process was scaled-down in a ratio of (b) (4). The acceptance criteria for (b) (4) were kept identical. The biochemical test results, such as test results for the (b) (4), are comparable between the proposed commercial-scale and the down-scale.

We noticed that the post-use integrity tests for the (b) (4) 35N, 20N, and 15N nanofilters at the proposed commercial-scale are stated as “(b) (4)” (with reference to page 14 of 21 in Report No. *IG_MP-000033_ING_v10* and page 17 of 24 in Report No. *IG_MP-000034_ING_v7* under the respective Section 3.2.P.3.3 *Description of manufacturing process and process controls*). To confirm the pore size distribution of the post-use nanofilters, we asked IG if a (b) (4) is performed for post-use integrity testing for (b) (4) 35N/20N nanofilters in the manufacture of Human Fibrinogen component, and for the (b) (4) 15N nanofilters in the manufacture of Human Thrombin component.

An IR was sent to IG on August 7, 2017, to which IG responded in Amendment #43 on September 8, 2017. IG’s response is summarized as follows:

The validation data provided by (b) (4), the manufacturer of (b) (4) filters, indicated that either the GPT or the (b) (4) test ((b) (4)) are qualified to be used for confirming the pore size distribution of the referenced nanofilters. Thus, IG uses (b) (4) to confirm the pore size distribution of the referenced post-use nanofilters.

Product reviewer’s comment: This response is acceptable.

- 3) Glycine precipitation: IG provided the qualification data of the down-scale system used for viral removal by glycine precipitation (*Study No. IG_ISVR-000167_ING*). In the study, the proposed commercial -scale process was scaled down in a ratio of (b) (4). The biochemical test results, including (b) (4)

(b) (4) of the third glycine precipitation step, are comparable between the down-scale and the proposed commercial-scale.

Product reviewer's comment: The down-scale systems used for viral clearance are representative of the proposed commercial-scale manufacturing process. Therefore, the viral clearance data generated from the down-scale systems can be used to evaluate the viral clearance capacity of the referenced manufacturing steps.

3.4.2.2. Viral clearance studies – Human Fibrinogen component:

The following viruses were selected to be used in the viral clearance studies:

- Relevant enveloped virus: HIV-1
- Model virus for enveloped DNA viruses including HBV: Pseudorabies virus (PRV)
- Model virus for enveloped RNA viruses: West Nile Virus (WNV) and Bovine viral diarrhea virus (BVDV)
- Relevant non-enveloped virus: HAV
- Model virus for B19V: Porcine parvovirus (PPV)

These viruses resemble viruses which may contaminate the Human Fibrinogen component, and represent a wide range of physico-chemical properties in the testing of the ability of the manufacturing process to eliminate viruses.

The materials used for virus clearance studies were tested for toxicity and interference with virus titration assays. The infectivity titer of each sample was determined by using either the *Spearman-Kärber* method or the *Poisson* distribution at 95% upper confidence limits. Viral clearance studies were performed by deliberately spiking samples collected at relevant manufacturing steps.

1) Solvent/Detergent treatment – Human Fibrinogen component

The viral clearance data on S/D treatment for the production of the Human Fibrinogen component are listed as follows:

- Validation of the inactivation of human HIV by treatment with S/D of a Fibrinogen solution (*Study No. IG_ANALYSIS-ISVR 2-99.117*)
- S/D step: viral validation study of the Fibrinogen production process: BVDV and PRV (*Study No. IG_UB-ISVR 0350300-006_ING*)
- Fibrinogen purification process. Validation of WNV inactivation during S/D treatment. Preliminary study of process material effects on virus and detector cell lines (*IG_BRL-ISVR AD41JV.026000.BUK*)

Based on the viral clearance studies above, the kinetics for the referenced four enveloped viruses are graphed as follows:

(b) (4)

As the data above show, the infectivity was below the LOD for WNV, BVDV, HIV-1, and PRV after the respective 15 min, 15 min, 30 min, and 120 min of S/D treatment using (b) (4) assays. Down-scale studies examining the S/D treatment, resulted in the following log reduction factors: HIV-1 (≥ 5.33), PRV (≥ 6.80), WNV (≥ 5.20), and BVDV (≥ 5.60). Studies of the viral clearance robustness of PRV were performed because PRV appears to be the most resistant model virus among the tested enveloped viruses. These studies indicated that even when using (b) (4) of the nominal concentration of S/D treatment at proposed commercial-scale and at aforementioned low temperature (b) (4), at least 6 log₁₀ viral clearance capacity was achieved.

Product reviewer's comment: IG provided extensive viral clearance studies on the S/D treatment stage of the manufacturing process of the Human Fibrinogen component. These include robustness studies under various conditions, (b) (4). Based on this information, we consider the S/D treatment a robust step for the inactivation of the referenced enveloped viruses.

- 2) Nanofiltration ((b) (4) 35N and 20N nanofilters (b) (4)) – Human Fibrinogen component

The capacity of the removal of enveloped and non-enveloped viruses was tested in a down-scale system for the nanofiltration step. IG also examined the critical process parameters in the robustness studies on nanofiltration for the clearance of HIV-1, PRV, WNV, BVDV, HAV, and PPV. The nanofilters ((b) (4) 35N and 20N) were challenged

with the (b) (4) and (b) (4), which were either equal or slightly over the upper limit of the proposed commercial parameters.

The robustness studies indicated that the nanofiltration step can achieve viral reduction of $\geq 5.57 \log_{10}$ for HIV-1, $\geq 6.09 \log_{10}$ for PRV, $\geq 4.51 \log_{10}$ for WNV, $\geq 4.53 \log_{10}$ for BVDV, and $5.22 \log_{10}$ for HAV. The studies using human B19V (infectivity) are considered experimental in nature. Therefore, viral clearance studies (infectivity) on PPV, a model virus of B19V, were performed. These studies indicated that the nanofiltration step can achieve viral reduction of $4.37 \log_{10}$ for PPV. The details on robustness are described in the following studies:

- Validation of the removal of HIV Type 1 by double nanofiltration ((b) (4) 35N and 20N) in the Fibrinogen manufacturing process (*Study No. IG_NL-ISVR 2-05.134*)
- Virus removal step by double nanofiltration through (b) (4) 35N and 20N filters; viral validation study of the Fibrinogen production process; PRV (*Study No. IG_UB-ISVR 0350300-046_ING*)
- Virus removal step by double nanofiltration through (b) (4) 35N and 20N filters; viral validation study of the Fibrinogen production process; BVDV (*Study No. IG_UB-ISVR 0350300-047_ING*)
- Fibrinogen purification process. Validation of WNV elimination during nanofiltration through (b) (4) 35N and 20N filters (*Study No. IG_BRL-ISVR AD41JW.028925*)
- Virus removal step by nanofiltration ((b) (4) 35N and 20N filters); viral validation study of the adhesive Fibrinogen production process; HAV (*Study No. IG_UB-ISVR 0350300-039_ING*)
- Virus removal step by nanofiltration through (b) (4) 35N and 20N filters; viral validation study of the adhesive Fibrinogen production process; PPV (*Study No. IG_UB-ISVR 0350300-038_ING*)

Product reviewer's comment: Robustness studies examining the nanofiltration ((b) (4)) step, using the referenced viruses were performed. Slightly (b) (4) have no substantial impact on the viral removal. Additionally, at least (b) (4) independent runs were conducted for each virus, which are consistent with the requirement of ICH Q5A guidance. All the data provided in these studies support the nanofiltration step ((b) (4) 35N and 20N nanofilters (b) (4)) as an effective stage for the removal of potential contamination of both enveloped and non-enveloped viruses.

3) Glycine precipitation – Human Fibrinogen component

Regarding non-enveloped viral clearance, other than nanofiltration, the studies on Glycine precipitation were performed. The studies indicated that the triple Glycine precipitation steps can achieve viral reduction of $5.21 \log_{10}$ for HAV, and $2.09 \log_{10}$ for PPV. Together with the viral clearance data generated from the nanofiltration step, the total reduction factor that was achieved was $10.43 \log_{10}$ for HAV and $6.46 \log_{10}$ for PPV.

3.4.3. Validation of viral clearance in selected steps of the Human Thrombin component manufacturing process

3.4.3.1. Down-scale validation – Human Thrombin component:

Similarly to the down-scale studies on Human Fibrinogen component, IG did not provide the down-scale factor relative to the proposed commercial-scale in each of the viral clearance studies. We asked IG to provide the relevant information in an IR dated August 7, 2017, and they responded in Amendment #43 on September 8, 2017. Their response is summarized as follows:

- S/D treatment: IG provided the qualification data of the down-scale systems used for viral inactivation by S/D treatment (*Study No. IG_ISVR 02-007_ING and IG_BRL-ISVR AD41JT.028925.BUK*). In these studies, the proposed commercial-scale process was scaled down in a ratio of (b) (4) (HIV, PRV, and BVDV) or (b) (4) (WNV). Additionally, the critical parameters including the (b) (4) were adjusted to the worst-case scenario. (b) (4)
- Nanofiltration (double (b) (4) 15N nanofilters (b) (4)): IG provided the data on the qualification of the down-scale system used for viral removal by nanofiltration (*Study No. IG_ISVR 000055_ING*). In the study, the proposed commercial-scale process was scaled down in a ratio of (b) (4). The acceptance criteria for (b) (4) before filtration, (b) (4) are identical. The results of the biochemical parameters such as (b) (4) in the filtrate are comparable between the proposed commercial-scale and the down-scale.
- Fraction I precipitation: IG provided the data on the qualification of the down-scale system used for viral clearance by Fraction I precipitation (*Study No. IG_ISVR-04-094_ING*). In the study, the proposed commercial-scale process was scaled down in a ratio of (b) (4). The test results of biochemical parameters including (b) (4) in the Fraction I supernatant are comparable between the down-scale and the proposed commercial-scale.
- SP-Sepharose XL ion-exchange chromatography: IG provided the data on the qualification of the down-scale system used for viral removal by SP-Sepharose XL ion-exchange chromatography (*Study No. IG_ISVR-000144_ING*). In the study, the proposed commercial-scale process was scaled down in a ratio of (b) (4). The test results of biochemical parameters including (b) (4) in the column eluate are comparable between the down-scale and the proposed commercial-scale.

Product reviewer's comment: These data demonstrate that each down-scale system used for viral clearance is representative of the commercial-scale manufacturing process. Therefore, the

viral clearance data generated from these down-scale systems can be used to evaluate the viral clearance capacity of the referenced manufacturing steps.

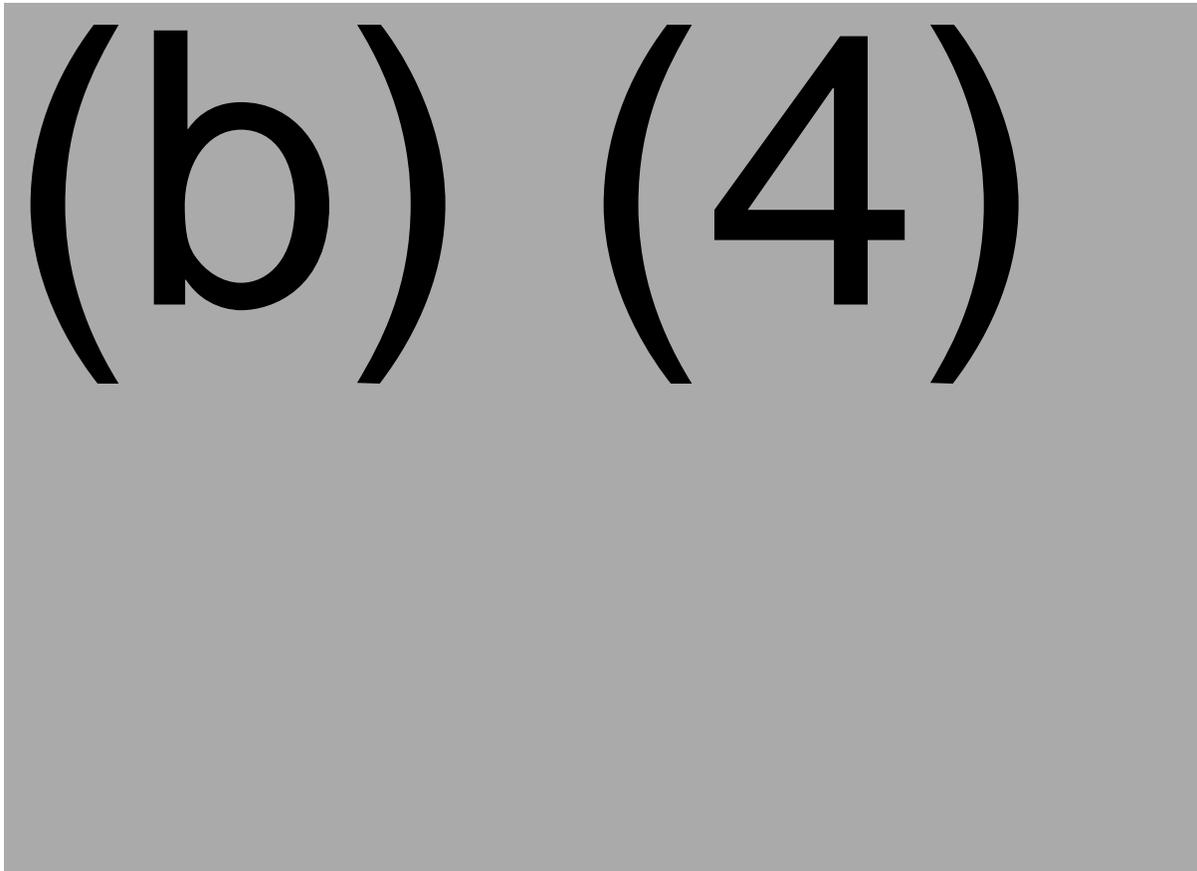
3.4.3.2. Viral clearance studies – Human Thrombin component:

1) Solvent/Detergent treatment – Human Thrombin component

The viral clearance data on S/D treatment for the production of Human Thrombin component are listed as follows:

- Validation of the inactivation of human HIV by treatment with S/D of a Thrombin solution (*Study No. IG_ANALYSIS-ISVR 2-01.110*)
- S/D step: viral validation study of the Thrombin production process: BVDV and PRV (*Study No. IG_UB-ISVR 0350300-011_ING*)
- Fibrinogen purification process. Validation of WNV inactivation during S/D treatment. Preliminary study of process material effects on virus and detector cell lines (*IG_BRL-ISVR AD41JT.026000*)

Based on the viral clearance studies above, the kinetics for the referenced four enveloped viruses are graphed as follows:



As the data above show, the infectivity was below the LOD for WNV, BVDV, HIV-1, and PRV after the respective 30 min, 30 min, 60 min, and 120 min of S/D treatment using (b) (4) assays. Down-scale studies on S/D treatment resulted in the

following log reduction factors, in parenthesis, for these viruses: HIV-1 (≥ 5.52), PRV (≥ 5.85), WNV (≥ 5.94), and BVDV (≥ 5.09). Additionally, the studies of robustness on PRV were performed. These studies indicated that even (b) (4) of the nominal concentration of S/D treatment at the proposed commercial-scale and aforementioned low temperature, at least 5.20 log₁₀ viral clearance capacity was achieved.

Product reviewer's comment: IG provided extensive viral clearance studies on S/D treatment that is a part of the manufacturing process in the production of Human Thrombin component. These include the robustness studies under the various conditions, such as (b) (4). Also, according to the requirements of the FDA guidance regarding viral safety, reproducible clearance was demonstrated in at least (b) (4) independent studies. Based on this information, we consider that S/D treatment is a robust step for the inactivation of the referenced enveloped virus.

2) Nanofiltration (double (b) (4) 15N nanofilters (b) (4)) – Human Thrombin component

The capacity of the removal of enveloped and non-enveloped viruses was tested at down-scale for the nanofiltration step. IG also examined the critical process parameters in the robustness studies on nanofiltration for the clearance of PPV. The (b) (4) 15N nanofilters were challenged with (b) (4), which was either nominal or at the upper limit of this parameter. The limit for (b) (4) at the proposed commercial-scale is set to be (b) (4).

The studies indicated that the nanofiltration step (double (b) (4) 15N nanofilters (b) (4)) can achieve viral reduction of $\geq 4.03 \log_{10}$ for HIV-1, $\geq 5.95 \log_{10}$ for PRV, $\geq 5.42 \log_{10}$ for WNV, and $\geq 4.93 \log_{10}$ for BVDV although the limitation of viral titration of these viruses. These studies also indicated that the nanofiltration step can achieve viral reduction of 6.56 log₁₀ for HAV and 6.14 log₁₀ for PPV. The details on viral clearance are described in the following studies:

- Validation of the removal of HIV Type 1 by double nanofiltration ((b) (4) 15N) (*Study No. IG_ANALYSIS-ISVR 2-02.101*)
- Virus removal step by double nanofiltration ((b) (4) 15N); viral validation study of the Thrombin production process; PRV (*Study No. IG_UB-ISVR 0350300-015_ING*)
- Virus removal step by double nanofiltration ((b) (4) 15N); viral validation study of the Thrombin production process; BVDV (*Study No. IG_UB-ISVR 0350300-016_ING*)
- Thrombin purification process. Validation of WNV elimination during nanofiltration through (b) (4) filters (*Study No. IG_BRL-ISVR AD41JU.028925.BUK*)
- Virus removal step by double nanofiltration ((b) (4) 15N); viral validation study of the Thrombin production process; HAV (*Study No. IG_UB-ISVR 0350300-014_ING*)
- Virus removal step by double nanofiltration ((b) (4) 15N); viral validation study of the Thrombin production process; PPV (*Study No. IG_UB-ISVR 0350300-013_ING*)

Product reviewer's comment: Robustness studies on the nanofiltration (double (b) (4) 15N nanofilters (b) (4)) step using PPV, the smallest virus in the tested ones,

were performed. Slightly (b) (4) has no substantial impact on the viral removal. All the data provided in these studies support that the nanofiltration step (double (b) (4) 15N nanofilters (b) (4)) is effective for the removal of potential contamination of both enveloped and non-enveloped viruses.

3) Fraction I precipitation – Human Thrombin component

The viral clearance studies on Fraction I precipitation were performed. The studies indicated that the Fraction I precipitation step can achieve viral reduction of 2.13 log₁₀ for PRV, 1.34 log₁₀ for BVDV, 2.78 log₁₀ for WNV, and 1.18 log₁₀ for HAV. Because the log reduction factor for both HIV and PPV was less than 1 log₁₀ from at least one of the respective (b) (4) experiments, the effectiveness of this step for the clearance of both HIV and PPV was excluded.

4) SP-Sepharose XL ion-exchange chromatography – Human Thrombin component

IG performed the studies on SP-Sepharose XL ion-exchange chromatography other than nanofiltration regarding non-enveloped viral clearance. The studies indicated that the SP-Sepharose XL ion-exchange chromatography step can achieve viral reduction of 4.61 log₁₀ for HAV, and 3.97 log₁₀ for PPV.

Product reviewer’s comment: Viral clearance studies for SP-Sepharose XL ion-exchange chromatography need to be performed with fresh resins as well as resins that have been used for the specified maximum number of cycles. However, IG did not specify the number of cycles for the resins used in the relevant viral clearance studies (*Study No. IG_ISVR-000156_ING and IG_ISVR-000161_ING*). If these studies were performed with fresh resins only, we do need IG to submit results from studies performed using resins that have undergone the maximum number of cycles for the clearance of HAV and PPV.

An IR was sent to IG on August 7, 2017, and the issue was also discussed with IG during the late-cycle meeting on 31 August 2017. IG confirmed that the resins used in all these studies were fresh resins. As shown in the viral clearance studies on nanofiltration, the manufacturing process of Human Thrombin component can achieve at least 6 log₁₀ reduction for non-enveloped viruses even that the SP-Sepharose XL ion-exchange chromatography step is excluded in the calculation. In the absence of the requested additional data for the SP-Sepharose XL ion-exchange chromatography step, it would not have significant impact on the product safety regarding non-enveloped virus clearance. (b) (4)

3.4.4. *Virus reduction summary – Fibrin Sealant (Human)*

Based on the viral clearance data provided in this BLA, the log reduction factors of the different manufacturing steps for the relevant and model viruses are summarized in Tables 1 and 2.

Table 1. Global virus reduction factors (log₁₀) for inactivation/removal of various viruses achieved by the manufacturing process of Human Fibrinogen component

Manufacturing step	Virus reduction factor (log ₁₀)*					
	Enveloped viruses				Non-enveloped viruses	
	HIV-1	PRV	WNV	BVDV	HAV	PPV
Glycine precipitations	n.d.	n.d.	n.d.	n.d.	5.21	2.09
S/D treatment	≥ 5.53	≥ 6.80	≥ 5.20	≥ 5.60	n.a.	n.a.
Nanofiltration	≥ 5.57	≥ 6.09	≥ 4.51	≥ 4.53	5.22	4.37
Global virus reduction factor (log ₁₀)	≥ 10.90	≥ 12.89	≥ 9.71	≥ 10.13	10.43	6.46

Table 2. Global virus reduction factors (log₁₀) for inactivation/removal of various viruses achieved by the manufacturing process of Human Thrombin component

Manufacturing step	Virus reduction factor (log ₁₀)*					
	Enveloped viruses				Non-enveloped viruses	
	HIV-1	PRV	WNV	BVDV	HAV	PPV
Fraction I precipitation	< 1.0	2.13	2.78	1.34	1.18	< 1.0
S/D treatment	≥ 5.52	≥ 5.85	≥ 5.94	≥ 5.09	n.a.	n.a.
SP-Sepharose XL chromatography	n.d.	n.d.	n.d.	n.d.	4.61	3.97
Nanofiltration	≥ 4.03	≥ 5.95	≥ 5.42	≥ 4.93	6.56	6.14
Global virus reduction factor (log ₁₀)	≥ 9.55	≥ 13.93	≥ 14.14	≥ 11.36	12.35	10.11

*: Reduction factor below 1 log₁₀ is not considered in calculating the overall virus reduction; n.d.: Not done; n.a.: Not applicable

Product reviewer’s comment: As described above, the drug product safety related to the potential viral contamination in the manufacturing processes is mainly guaranteed through these viral clearance steps other than the control of the potential virus loading in the manufacturing plasma pool. These results are sufficient to support the effectiveness of viral clearance in the commercial manufacturing process of Fibrin Sealant (Human).

4. Recommendation

The safety of non-viral adventitious agents including bacteria, fungi, and mycoplasma is well controlled by validated cleaning/sanitization procedures, in-process controls, filtration steps including (b) (4) sterile filtration, and implementation of release tests of Sterility and

Endotoxins in the final product of Fibrin Sealant (Human). The safety of adventitious viruses is well controlled in the manufacturing process of Fibrin Sealant (Human): S/D treatment proves effective in inactivating the enveloped viruses; Nanofiltration is confirmed to be a critical step for the removal of both enveloped and non-enveloped viruses; Additionally, glycine precipitation in the manufacture of Human Fibrinogen component contributes to the clearance of non-enveloped viruses; Fraction I precipitation and SP-Sepharose XL chromatography in the manufacture of Human Thrombin component contribute to the enveloped and/or the non-enveloped virus clearance. The viral safety in the manufacturing processes is mainly guaranteed through these viral clearance steps other than the control of the potential virus loading in each manufacturing plasma pool. The measures taken by IG to control adventitious agents in the manufacture of *Fibrin Sealant (Human)* are acceptable.

Therefore, we recommend approval of the BLA with the following PMC committed by IG:

Instituto Grifols, S.A. commits to providing results from small-scale studies for the (b) (4) [REDACTED]. The final results will be submitted as a “Postmarketing Study Commitment - Final Study Report” by December 31, 2018.

Appendix: History of information requests

Date of Information Request	Communicated Review Issues between the FDA and IG	Amendment/ Date of Response
April 18, 2017	<p>1. <i>To better estimate the potential virus load in the starting material, please submit the Limit of Detection of each of the Nucleic Acid Tests (NAT) used for detection of Human Immunodeficiency Virus, Hepatitis B Virus, Hepatitis C Virus, Hepatitis A Virus, or Parvovirus B19 (B19V) in the (b) (4) as well as the manufacturing plasma pool.</i></p> <p>2. <i>In Section 3.2.S.2.3. Control of Materials (Human Fibrinogen or Human Thrombin), you stated that the (b) (4) and manufacturing plasma pools have been tested and found to be non-reactive for viral markers of infection, including B19V.</i></p> <p><i>Please provide the acceptance limits for the level of B19V DNA in the (b) (4) and manufacturing pool, and the results of all the plasma pools tested so far based on your quantitative NAT for B19V.</i></p>	Amendment #26 April 25, 2017
August 7, 2017	<p>3. <i>Please provide the down-scale factor relative to the commercial-scale process in each of the viral clearance studies, and demonstrate that the scale-down studies are representative of commercial manufacturing.</i></p> <p>4. <i>Viral clearance studies for column chromatography need to be performed with fresh resins as well as resins that have been used for the specified maximum number of cycles. Please specify the number of cycles for the resins used in viral clearance studies IG_ISVR-000156_ING and IG_ISVR-000161_ING. If these studies were performed with fresh resins only, please submit results from studies performed using resins that have undergone the maximum number of cycles for the clearance of relevant and model viruses.</i></p> <p>5. <i>Please confirm if the (b) (4) test is used in post-use integrity test for the (b) (4) 35N/20N nanofilters used in the manufacture of Human Fibrinogen, and for the (b) (4) 15N nanofilters used in the manufacture of Human Thrombin.</i></p>	Amendment #43 September 8, 2017
	6. <i>The alternative due date (i.e., December 31, 2018)</i>	

<p>October 3, 2017</p>	<p><i>proposed by Instituto Grifols, S.A. for submission of the “Postmarketing Study Commitment – Final Study Report” is acceptable. Our updated language for a Postmarketing Commitment (PMC) with regard to BL 125640 is as follows:</i></p> <p><i>Instituto Grifols, S.A. commits to providing results from small-scale studies for the (b) (4)</i></p> <p><i>[REDACTED]</i></p> <p><i>. The final results will be submitted as a “Postmarketing Study Commitment - Final Study Report” by December 31, 2018.</i></p>	<p>Amendment #53 October 4, 2017</p>
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