



MEMORANDUM

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

To: File (STN 125506/0)
Pratibha Rana, Regulatory Project Manager, RPMB/DBA/OBRR

From: Ze Peng, PhD, LH/DH/OBRR

Through: Mark Weinstein, PhD, Assoc. Dep. Dir. for Science, OBRR
Basil Golding, MD, Division Director, DH/OBRR

Subject: Final Review of Viral Safety information in Bio Products Laboratory
Limited's original BLA for Coagulation Factor X (Human)

Cc: Mikhail V. Ovanesov, PhD, Committee Chair, LH/DH/OBRR

This memorandum summarizes the review of Adventitious Agents Safety Evaluation (Section 3.2.A.2) in an original Biologics License Application (BLA) under STN 125506 submitted by Bio Products Laboratory Limited (BPL) for Coagulation Factor X (Human) (FX). The proposed proprietary name of this product is *Replafacten*. In general, the measures taken by BPL to control adventitious agents in the manufacture of *Replafacten* are acceptable. Therefore, I recommend approval of the BLA under STN 125506/0.

Executive Summary

Evaluation of Safety Regarding Adventitious Agents

BPL manufactures the *Replafacten* drug product (DP) 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 ((b) (4)), and in-process filtration steps including ((b) (4)) sterile filtration. The final container of *Replafacten* is further guaranteed to be free of non-viral adventitious agents by the testing for Sterility and Endotoxins.

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. The routine cleaning of equipment with ((b) (4)) will remove/inactivate

potential TSE agent contamination. Furthermore, the manufacturing steps including (b) (4) nanofiltration (pore size, 15 nm) may contribute to the removal of potential TSE agent contamination.

The potential viral load in the starting material is well controlled in the manufacture of *Replafacten*. This product is manufactured using U.S. Source Plasma (21 CFR 640.60), which is obtained from FDA-licensed U.S. plasma collection centers. Plasma donations used for *Replafacten* have to be tested negative for serological markers (mandatory testing for Hepatitis B surface Antigen (HBsAg), antibodies against Human Immunodeficiency Virus (HIV)-1/2 and Hepatitis C Virus (HCV)). Mini-pools of donations are tested by nucleic acid technique (NAT)^{(b) (4)} for the presence of genomic material of Hepatitis A Virus (HAV), Hepatitis B Virus (HBV), HCV, HIV-1, and human parvovirus B19 (B19V). Donations reactive to HAV, HBV, HCV, HIV-1 or with a high titer ^{(b) (4)} for a single donation) of B19V are excluded from further manufacture. In-process controls are performed on the manufacturing pools. Each pool is tested to be negative for HBsAg and anti-HIV-1/2. Also, manufacturing pools are non-reactive for HAV, HBV, HCV, and HIV-1. The limit for B19V in the manufacturing pools ^{(b) (4)} per pool) is set not to exceed 10⁴ IU/mL.

Additionally, the potential of viral contamination of *Replafacten* is mitigated by three dedicated viral clearance steps: Solvent/Detergent (S/D) treatment ^{(b) (4)}, terminal dry heat (TDH) treatment (80°C for 72 hours), and ^{(b) (4)} nanofiltration. BPL has evaluated these three steps in down-scale studies. The viruses selected in the studies include enveloped viruses, HIV-1, Infectious bovine rhinotracheitis (IBR, bovine herpesvirus model for enveloped DNA viruses including HBV), Herpes simplex virus type 1 (HSV-1, model virus for large enveloped DNA viruses), Sindbis virus (SBV, model virus for HCV), Bovine viral diarrhea virus (BVDV, model virus for enveloped RNA viruses), West Nile Virus (WNV), and non-enveloped viruses, HAV, Canine parvovirus (CPV, model virus for B19V), and B19V. These viruses resemble viruses which may contaminate the *Replafacten* DP, and represent a wide range of physico-chemical properties in the testing of the ability of the manufacturing process to eliminate viruses. Down-scale studies on the relevant steps resulted in the following global log reduction factors, in parenthesis, for these viruses: HIV-1 (> 16.9), IBR (> 5.3), HSV-1 (> 14.7), SBV (6.0), BVDV (> 14.8), WNV (4.9), HAV (> 11.1), CPV (8.5), and B19V (> 5.9). These results are sufficient to support the effectiveness of viral clearance in the commercial manufacturing process.

Background

Replafacten is a plasma-derived, purified concentrate of freeze-dried human FX protein. This product also contains a trace amount of Factor II (FII), and factor IX (FIX) but these amounts of FII and FIX have no therapeutic value. *Replafacten* is manufactured in BPL's facility located at Elstree, United Kingdom. This product is supplied as a powder

for reconstitution with water for injection, and is used only for intravenous injection. *Replafacten* contains 250 or 500 IU of FX activity per vial.

The plasma used for the manufacture of *Replafacten* is obtained from FDA-licensed plasma donation centers according to 21 CFR 640.30 (Plasma) and 21 CFR 640.60 (Source Plasma). There are three dedicated steps for viral clearance in the manufacturing process, which include two viral inactivation steps, i.e., S/D treatment and TDH treatment, and one viral removal step, i.e., (b) (4) nanofiltration.

Summary of Review

Flow chart of the manufacturing process of Replafacten



Product reviewer's comment: As highlighted in the above flow diagram, there are three dedicated virus clearance steps in the manufacturing process, which are consistent with recommendation from FDA. These steps are considered to contribute to the improvement of viral safety of this product provided that these steps are completely validated for viral clearance.

Evaluation of non-viral adventitious agent safety

BPL manufactures the *Replafacten* DP 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 ((b) (4)), and in-process filtration steps including (b) (4) sterile filtration. *Replafacten* is further guaranteed to be free of non-viral adventitious agents by the testing of Sterility and Endotoxins in the final container. Therefore, the measures taken by BPL to control non-viral adventitious agents in the manufacture of *Replafacten* are acceptable.

(b) (4)

To minimize the risk of TSE agent transmission, 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. In addition, BPL performed TSE agent validation studies regarding the following *Replafacten* manufacturing steps at down-scale:

(b) (4)

(b) (4)

Evaluation of virus safety

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 *Replafacten* 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 for the absence of HBsAg, antibodies against HCV, and HIV-1/2. Thus, donor selection is performed in accordance with the requirements of the 21 CFR and the respective FDA guidelines.

Also, BPL specified that human plasma used for the manufacture of *Replafacten* is Source Plasma (21 CFR 640.60) as indicated in the relevant document collected during the on-site inspection (19-25 October 2013). I defer the review of the qualification of this type of plasma in the manufacture of *Replafacten* to Dr. Mikhail V. Ovanesov.

2. Testing the manufacturing pool for the absence of contaminating infectious viruses

The starting material, human plasma used in the manufacture of *Replafacten* will be tested by (b) (4) using NAT/(b) (4) assay. The detailed information was provided in the relevant documents collected during the on-site inspection, and summarized as follows:

- 1) Mini-pool (≤ 512 donations): Each pool is tested for the absence of viral genome of HAV, HBV, HCV, and HIV-1.
- 2) Manufacturing plasma pool: Each pool is tested to be negative for HBsAg and anti HIV-1/2. These pools can be released only if they are also non-reactive for HAV, HBV, HCV, HIV-1, and have titer of B19V $\leq 10^4$ IU/mL. The sensitivity of each NAT^{(b) (4)} assay performed by ^{(b) (4)} is (b) (4) for HIV, (b) (4) for HBV, (b) (4) for HCV, (b) (4) for HAV, and (b) (4) for B19V. The sensitivity of each NAT assay performed by ^{(b) (4)} is similar to that from the multiple test kit performed by (b) (4) e.g., for the multiple test kit (b) (4) the sensitivity is (b) (4) for HIV-1 group (b) (4) for HIV-1 group (b) (4) for HIV-2, (b) (4) for HBV, (b) (4) for HCV, for multiple test kit (b) (4), the sensitivity is ^{(b) (4)} for HAV, and (b) (4) for B19V.

Product reviewer's comment: BPL stated that each mini-pool used for the manufacture of *Replafacten* DP is tested for B19V. However, they did not provide the limit for the titer of B19V in the mini-pool that would exclude the pool from further manufacture. I will request this information from BPL to complete the evaluation of the potential viral loading in the manufacturing pool.

This comment was sent to BPL on 6 February 2014, and they responded in an amendment on 12 February 2014. BPL confirmed in this amendment that B19V testing in mini-pool is for exclusion of any single donation to have ^{(b) (4)} of the titer of B19V. Considering that the limit of B19V in the manufacturing plasma pool will be not more than 10^4 IU/mL, this response is acceptable.

3. *Selected steps in the manufacturing process of Replafacten were validated for the capacity to inactivate viruses*

Down-scale validation:

There are three dedicated virus clearance steps introduced in the *Replafacten* manufacturing process, including S/D treatment ((b) (4)) nanofiltration, and TDH treatment (80°C for 72 hours). To support the following viral clearance studies, I will ask BPL to provide data to demonstrate that each down-scale system (i.e., S/D treatment, (b) (4) nanofiltration, and TDH treatment) used for the viral clearance studies qualifies as being representative of the full-scale system.

This comment was sent to BPL on 6 February 2014, and they responded in an amendment on 12 February 2014. Their response is summarized as follows:

- S/D treatment: BPL provided the qualification data on the down-scale system used for viral inactivation by S/D treatment (*Study Nos. V0785 and V0998*). In

these studies, the test results of critical parameters including the concentration of (b) (4) are comparable between down-scale and full-scale.

- (b) (4) nanofiltration: BPL provided the qualification data on the down-scale system used for viral removal by (b) (4) nanofiltration (*Study Nos. V0893, V0903, and V0905*). In these studies, the filtration was carried out in a down-scale system using (b) (4).
These studies showed that the protein recovery (b) (4) was not significantly reduced during the nanofiltration with increased FX loading and pressure.
- TDH treatment: Similarly, BPL provided the qualification data on the down-scale system used for viral inactivation by TDH treatment (*Study Nos. V0942, V0955, and V0966*). In these studies, the test results of critical parameters including (b) (4) are comparable between down-scale and full-scale. FX potency recovery after the TDH treatment is within the historical range generated at full-scale ((b) (4)). Residual moisture is also comparable between down-scale and full-scale.

Product reviewer's comment: These data demonstrated that the down-scale system used for the respective viral clearance is representative of the full-scale manufacturing process. Therefore, the viral clearance data generated using these down-scale systems can be used for the evaluation on the viral clearance capacity of the referenced manufacturing steps. This response is acceptable.

Viral clearance studies:

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

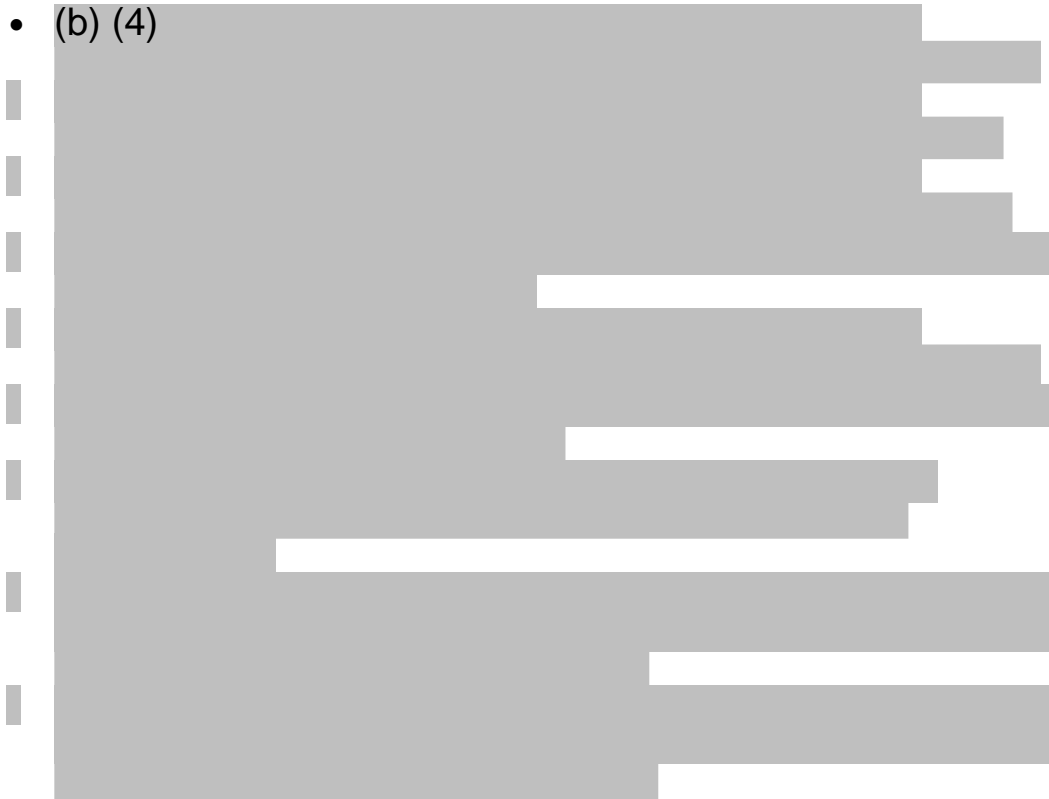
- Relevant enveloped viruses: HIV and WNV
- Model virus for enveloped DNA viruses: HSV-1 and IBR
- Model virus for HCV: SBV and BVDV
- Relevant non-enveloped viruses: HAV and B19V
- Model virus for B19V: CPV

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

Inhibition of virus infection due to cytotoxicity by the test samples did not occur when all test samples were diluted at least (b) (4) before inoculating the cell culture. Viral clearance studies were performed by deliberately spiking samples collected at the relevant manufacturing steps.

1) Solvent/Detergent treatment

As supported by the data provided in the viral clearance studies, log reduction factors, in parenthesis, for these viruses: HIV-1 (> 4.6), WNV (4.9), HSV-1 (> 5.4), IBR (b) (4), SBV (b) (4), and BVDV (> 5.1) for the S/D treatment. Moreover, BPL did the following robustness studies for WNV, HSV-1, IBR, SBV, and BVDV viruses examining the critical process parameters for S/D treatment.

- (b) (4)
- 

Based on the above studies, the kinetics for the referenced 6 enveloped viruses is graphed as follows:

(b) (4)

(b) (4)

As the data shown above, the robustness of the S/D treatment step was demonstrated under the worst case scenario such as (b) (4) on their limits set in the in-process control.

Product reviewer's comment: BPL performed extensive viral clearance studies on the S/D treatment that is a part of the manufacturing process in the production of *Replafacten*. 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 two independent studies. (b) (4)

Based on this information, I consider that the S/D treatment is a robust step for the inactivation of the referenced enveloped virus.

- 2) (b) (4) Nanofiltration

The capacity of the removal of enveloped and non-enveloped viruses was tested at down-scale for (b) (4) nanofiltration. BPL also examined the critical process parameters in the robustness studies on (b) (4) nanofiltration for the clearance of BVDV, HSV-1, HAV, CPV, and B19V viruses. The filters were



The studies indicated that (b) (4) nanofiltration can achieve viral reduction of $> 6.8 \log_{10}$ for HIV, $5.8 \log_{10}$ for HSV-1, $>4.5 \log_{10}$ for BVDV, and $> 5.0 \log_{10}$ for HAV. Although BPL achieved $5.9 \log_{10}$ B19V reduction (PCR) in the study on (b) (4) nanofiltration, they did not provide an infectivity study on B19V. The studies using human B19V (infectivity) are considered experimental in nature. Thus, BPL also performed viral clearance studies (infectivity) on CPV ($4.3 \log_{10}$), a model virus of B19V. The details on robustness are described in the following studies:

- Removal of CPV, B19V, and HAV from FX intermediate by (b) (4) filtration, (b) (4) (Study report No. V0903)
- Removal of BVDV from FX intermediate by (b) (4) filtration, (b) (4) (Study report No. V0905)
- Removal of HSV-1 from FX intermediate by (b) (4) filtration, (b) (4) (Study report No. V0907)
- Removal of small non-enveloped viruses from FX intermediate by (b) (4) filtration, (b) (4) on CPV and HAV removal (Study report No. V0957)
- Removal of small non-enveloped viruses from FX intermediate by (b) (4) filtration, (b) (4) on B19V removal (Study report No. V0968)

Product reviewer's comment: BPL performed robustness studies on (b) (4) nanofiltration using the referenced viruses. The critical parameters such as (b) (4) have no substantial impact on the viral removal. Additionally, at least two independent studies were conducted for each virus, which are consistent with the requirement of ICH Q5A guidance. All the data provided in these studies support that (b) (4) nanofiltration step is effective for the removal of potential contamination of both enveloped and non-enveloped viruses.

3) Terminal dry heat treatment

When BPL performed the viral clearance studies on TDH treatment at down-scale, they also included the lyophilization step. They only listed TDH treatment in the labeling because the viral inactivation capacity for lyophilization is very limited. The studies indicated that the combined steps including TDH treatment can achieve 5.5 log₁₀ of HIV-1 reduction, 3.5 log₁₀ of HSV-1 reduction, and > 5.2 log₁₀ of BVDV reduction. The combined steps including TDH treatment also had an inactivation capacity for non-enveloped viruses such as HAV (> 6.1 log₁₀), and CPV (4.2 log₁₀), a virus known to be resistant to physico-chemical treatment.

To demonstrate whether the critical parameters including (b) (4) have a potential effect on the efficiency of TDH treatment, BPL performed robustness studies on the combined steps including TDH treatment for the clearance of HSV-1, BVDV, HAV, and CPV. In these robustness studies, the residual moisture in the vials is either (b) (4) before TDH treatment (the level of residual moisture at the manufacturing scale is (b) (4) as stated in the virus validation studies). The data showed that there was no substantial difference for the kinetics of each referenced virus inactivation (i.e., HSV-1, BVDV, HAV, and CPV). Additionally, the temperature used in these robustness studies was (b) (4), around or even lower than the lower acceptable limit of the TDH temperature, and thus represents a worst case scenario.

Product reviewer's comment: The viral clearance studies performed on the combined steps including TDH treatment indicated that TDH treatment can effectively inactivate the referenced viruses including HIV, HSV-1, BVDV, HAV, and CPV. Together with the data from the robustness studies on these viruses, it supports that TDH treatment is a robust step for both enveloped and non-enveloped virus inactivation.

However, during the validation of the lyophilization step for viral inactivation (with reference to Appendixes 15-17), some reduction factors are (b) (4), which should not be included in the cumulative log reduction factors in accordance with ICH guideline Q5A. Therefore, I will ask BPL to verify if the relevant information in the viral reduction table of the prescribing information (PI) is correctly represented. If not, they should revise the PI accordingly.

This comment was sent to BPL on 8 November 2013 and 6 February 2014, and they provided the similar response on 13 December 2013 and 12 February 2014, respectively. Their response is summarized as follows:

BPL's response: A virus inactivation step comprising lyophilization followed by heat-treatment at 80°C for 72 hours is included in the *Replafacten* manufacturing process. This complete process step was validated for virus inactivation by determining the total inactivation obtained over the combined total process, i.e., lyophilization followed by heat-treatment. These total inactivation values are quoted in the virus inactivation summary tables and in the PI.

An additional analysis of the virus inactivation during the lyophilization step alone was performed, in order to understand the mechanism(s) of action. This data subset is also presented in the tables of Appendices 15-17, alongside the overall data. In those cases where virus inactivation across lyophilization was (b) (4), it could be inferred that freeze-drying made little contribution to the virus inactivation seen by the total experimental process step of lyophilization and heat-treatment. Thus, BPL concluded that the relevant information in the viral reduction table of the PI remains correct.

Product reviewer's comment: I noticed that some reduction factors are (b) (4) during the validation of the lyophilization step for viral inactivation (with reference to Appendices 15-17). However, the claimed TDH treatment was validated for virus inactivation by the combined two steps, i.e., lyophilization followed by TDH treatment. The viral titer in the samples at the time point of 72 hours during TDH treatment reflects the total viral clearance capacity of both referenced steps. Considering this reason, it is acceptable for BPL to use total viral reduction data for the combined steps (even some reduction factors are (b) (4) for the lyophilization step) after I consulted Dr. Farshid.

Virus reduction claimed

Based on the viral clearance data provided in this submission, BPL listed log reduction factors of the different manufacturing steps for relevant and model viruses in the following table.

Global virus reduction factors (log₁₀) for inactivation of various viruses achieved by the *Replafacten* manufacturing process

Manufacturing step	Virus reduction factor (log ₁₀)*								
	Enveloped viruses						Non-enveloped viruses		
	HIV	IBR	HSV-1	SBV	BVDV	WNV	HAV	CPV	B19V
S/D treatment	> 4.6	> 5.3	> 5.4	6.0	> 5.1	4.9	NT	NT	NT
15N nanofiltration	> 6.8	NT	5.8	NT	> 4.5	NT	> 5.0	4.3	> 5.9
TDH treatment	5.5	NT	3.5	NT	> 5.2	NT	> 6.1	4.2	NT
Total virus reduction factor (log ₁₀)	> 16.9	> 5.3	> 14.7	6.0	> 14.8	4.9	> 11.1	8.5	> 5.9

*: Reduction factor below (b) (4) is not considered in calculating the overall virus reduction; NT.: Not tested

Product reviewer's comment: As described above, S/D treatment proves effective in inactivating these enveloped viruses. The data also confirmed that the capacity of the TDH treatment step to effectively inactivate various sizes of enveloped viruses. Furthermore, the TDH treatment step proves to be effective in the inactivation of non-enveloped viruses such as HAV, and CPV. Additionally, (b) (4) nanofiltration was demonstrated to be a critical step for the removal of both enveloped and non-enveloped viruses. In general, the viral safety in the manufacturing process is mainly guaranteed through these three dedicated viral clearance steps.

The studies using human B19V (infectivity) are considered experimental in nature. I will ask BPL to remove the B19V data (b) (4) from the virus reduction factor table, and include a description of the B19V study in a footnote to the table.

This comment was sent to BPL on 6 February 2014, and I received their response on 12 February 2014. In their response, BPL agreed with FDA, and B19V data is now described as a footnote to the virus reduction factor table, and the PI has been revised accordingly. Therefore, this response is acceptable.

Recommendation

The safety of non-viral adventitious agents including bacteria, fungi, and mycoplasma is well controlled through the use of validated cleaning/sanitization procedures, in-process controls, filtration steps including (b) (4) sterile filtration, and release tests of Sterility and Endotoxins in the final container of *Replafacten*. The safety of adventitious viruses is well controlled through the manufacturing process of *Replafacten*, including three dedicated virus inactivation steps, S/D treatment, (b) (4) nanofiltration, and TDH treatment. Enveloped viruses are inactivated by both S/D treatment and TDH treatment, whereas non-enveloped viruses are mainly inactivated by TDH treatment. Enveloped and non-enveloped viruses are also removed by the use of (b) (4) nanofiltration. The measures taken by BPL to control adventitious agents in the manufacture of *Replafacten* are acceptable. Therefore, I recommend approval of the BLA under STN 125506/0.