



FOOD AND DRUG ADMINISTRATION
CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

MEMORANDUM

Final CMC review

To: File (STN BL 125506/0) & Pratibha Rana

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Subject: Final CMC Review of BPL's BLA for Coagulation Factor X (Human)

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1. Executive Summary

On 10 July 2013, Bio Products Laboratory Limited (BPL) submitted an original biologics license application (BLA) for Coagulation Factor X (Human) [FACTOR X]. The FDA granted this product Orphan Drug status (No. 07-2469) on 8 November 2007, Fast Track designation on 12 April 2012, and Priority Review for this BLA on 6 September 2013.

The product was developed as a replacement therapy to treat hereditary Factor [F] X deficiency, a rare bleeding disorder, for which no specific coagulation factor replacement therapy is currently available. The product contains a human Coagulation FX concentrate indicated for the control and prevention of bleeding episodes as well as for peri-operative management in adults and children (aged 12 years and over) with a hereditary FX deficiency.

Manufacturer

BPL is a well-established plasma fractionator and manufacturer of many plasma-derived products listed below:

- *Coagulation Factors and Inhibitors:* 8Y (Dried FVIII Fraction, type 8Y. Contains vWF, FVIII and (b) (4) Optivate (Human FVIII); Replenine-VF (high purity FIX); FXI

- concentrate (Orphan Drug, no brand name); (b) (4) (not licensed, no brand name)
- *Immunoglobulins*: Gammaplex (sterile liquid of 5% w/v normal immunoglobulin); Subgam (Human normal immunoglobulin solution (b) (4)); Vigam (sterile liquid of 5 g% normal immunoglobulin)
 - *Hyper-immunoglobulins*: D-Gam, Human Anti-D Immunoglobulin (RhoD IGIM); Human Varicella-Zoster Immunoglobulin; Human Tetanus Immunoglobulin; Human Rabies Immunoglobulin; Human Hepatitis B Immunoglobulin
 - *Human Albumin Solution*: Zenalb

Of these products, only Gammaplex 5% is currently licensed in the USA. (b) (4)

Final drug product

FACTOR X is a sterile, (b) (4) freeze-dried concentrate of human FX, presented as two nominal dose sizes of 250 International Units (IU) and 500 IU of FX. **I recommend labeling this product with actual, not nominal potency.** There is no manufacturing overage. After reconstitution with sterilized Water for Injection (sWFI), FACTOR X forms a clear, colorless solution.

The two dose sizes contain the same concentration of FX active ingredient (about 100 IU/mL) and formulation chemicals upon reconstitution. FX concentration is approximately 100-fold greater than that in normal plasma. Dose sizes differ only in the corresponding volumes at the point of fill and the point of use, e.g., 2.5 mL sWFI is supplied with the 250 IU dose, and 5 mL sWFI is supplied with the 500 IU dose.

A Mix2Vial device (510(k) number: K031861) is also supplied. The device is a sterile, non-pyrogenic, single-use fluid transfer device that allows quick transfer of sWFI to the FACTOR X freeze-dried product, and of the reconstituted FACTOR X product into a syringe for administration.

The composition of the FACTOR X product is described in Table 1 below.

Table 1: Composition of FACTOR X inactive ingredients

	Function	Reference	Quantity		
			per mL	per 250 IU vial	per 500 IU vial
Human FX	Active ingredient	BPL	100 IU	250 IU	500 IU
Inactive Ingredients					
Citric acid [a]	(b) (4)				
(b) (4)					
(b) (4)					
phosphate [a]					
Sodium chloride [a]	(b) (4)				
Sucrose [c]					
Water for Injections					

[a] Calculated from the product specification cation concentration.

[b] Calculated from formulation buffer composition.

[c] Calculated from product specification.

The impurities that are controlled by the release assays are shown in Table 2 below.

Table 2: Impurities

Impurities	Upper limit in weight per mL [a]	Source of impurity
Factor II	NGT 1 IU/mL	Residual protein components from plasma
Factor IX	NGT 1 IU/mL	
Factor Xa and IXa	N/A[b], NAPTT (b) (4)	
Thrombin	N/A[b], FCT (b) (4)	
(b) (4)	no specification[c]	
(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	
(b) (4)	(b) (4)	
(b) (4)	(b) (4)	(b) (4)
[a] Calculated from upper limits in the specification		
[b] Enzymatic thrombogenic impurities are controlled by non-quantitative functional coagulation tests NAPTT and FCT.		
[c] (b) (4)		

The specification for FACTOR X final drug product is shown in Table 3 below.

Table 3: FACTOR X Final Drug Product Specification

Test	Compliance	Test Limits
Characteristics		
Description of freeze-dried plug	BPL	Smooth white plug
Moisture, (b) (4)	BPL	(b) (4)
Solubility at (b) (4)	BPL	(b) (4)
Appearance of solution	BPL	Colorless, clear or slightly opalescent solution.
(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)
Stability at (b) (4)	BPL	(b) (4)
Identity	BPL	Product complies with limits of factor X assay
Biological Safety Tests		
Sterility test	BPL	Pass
Bacterial Endotoxin Test, (b) (4)	BPL	(b) (4)
General Safety Test	BPL	Pass
Purity/Specific Function		
Factor X activity, IU/mL	BPL	80 - (b) (4)
Factor X per vial, IU/vial	BPL	200 - (b) (4) (250 IU dose) 400 - (b) (4) (500 IU dose)
(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)
Total Protein, g/L	BPL	(b) (4)
Specific activity, IU/mg protein	BPL	(b) (4)
NAPTT (b) (4)	BPL	(b) (4)
NAPTT (b) (4)	BPL	(b) (4)

FCT (b) (4)	BPL	(b) (4)
Excipients		
Chloride, (b) (4)	BPL	(b) (4)
Phosphate, (b) (4)	BPL	(b) (4)
Citrate (b) (4)	BPL	(b) (4)
Sucrose (b) (4)	BPL	(b) (4)
Sodium, (b) (4)	BPL	(b) (4)
Impurities		
Factor II, IU/ml	BPL	NGT 1
Factor IX, IU/ml	BPL	NGT 1
(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)
NGT, Not Greater Than NLT, Not Less Than LT, Less Than		

I propose the following changes to the specifications:

1. Factor X activity per vial”
 - a. (b) (4) of nominal activity at release;
 - b. (b) (4) of labeled activity during the shelf-life of the product.
2. (b) (4) as identity & purity test:
 - a. (b) (4) comparability with reference (reference should be prepared from a FACTOR X batch)

Analytical methods have been reviewed by the laboratory of Dr. Lokesh Bhattacharyya (DBSQC/OCBQ) and Dr. Andrey Sarafanov (LH). Only a few methods have been described and validated adequately for use in quality control lot release tests: Determination of Chloride, Determination of Phosphate, Determination of (b) (4) Determination of (b) (4) Characteristics, Solubility, and Appearance of Reconstituted Solution. The remaining tests, including Potency and purity-indicating methods are not properly validated despite several rounds of information requests (IRs) and amendments with additional data provided by BPL during this review cycle.

Manufacturing process

FACTOR X is purified from Source Plasma of US origin at the FDA-licensed multi-product manufacturing facility that uses only US plasma. Plasma for FACTOR X manufacture is collected by FDA-licensed suppliers in accordance with the CFR. There are three dedicated virus clearance steps (solvent/detergent treatment, virus filtration, and terminal dry heat treatment).

The FACTOR X manufacturing process comprises of three previously established steps, which yield a FX-enriched intermediate (see Figure 1). The previously established steps have been part of the routine fractionation of plasma pools for the extraction and manufacture of plasma proteins **for more than 15 years**. They were introduced before the industry and regulators had developed formal strategies for process validation. The dedicated FACTOR X process steps have been operated at full scale at BPL’s GMP facility for the manufacture of clinical trial material since 2007.

(b) (4)

A pre-license inspection (PLI) was conducted on 21-25 October of 2013 by Drs. Randa Melhem (DMPQ) and Ze Peng (LH product reviewer). The PLI resulted in seven observations documented in Form FDA 483. The 483 observations included deficiencies in process validation, analytical method validation, (b) (4) conditions and documentation, validation of the lyophilization process, validation of cleaning and sterilization (b) (4) of lyophilizers, and Grade (b) (4) monitoring under dynamic conditions.

Process validation was conducted in 2009 prior to the initiation of the clinical trials. The exercise was not successful as evidenced by the high number of deviations observed. Results of continued process validation studies demonstrate that the existing in-process and release specifications are able to control the quality of the final product through the rejection of batches of intermediates that are outside of the set specifications. Poor state of process validation is evidenced by the need to terminate a large number of batches (5 out of (b) (4) batches manufactured after process validation were rejected because of different manufacturing deviations).

In response to the deficiencies identified during the PLI, the company initiated a new process validation exercise. The projected day of completion is 30 June 2014.

Stability:

All stability batches met the specifications at +5°C, +25°C and +30°C for up to 36 months. Therefore, the proposed shelf life of 36 months at +2°C to +30°C is acceptable.

Recommendation:

Many critical elements of the manufacturing process are not fully validated, which include analytical methods, cleaning, process performance and lyophilization. These deficiencies have been communicated to BPL, and BPL has initiated additional validation studies according to advices provided by FDA reviewers. BPL estimated that these studies will be completed on 30 June 2014,

which fails to meet the action due date for this BLA. Therefore, I recommend issuing a Complete Response letter.

2. Physicochemical and biological properties of FACTOR X

2.1. *Physicochemical properties*

FACTOR X is a (b) (4) freeze-dried powder which is readily soluble ((b) (4)) in sWFI. After reconstitution, FACTOR X is clear and colorless and contains a low concentration of protein ((b) (4)) stabilized with buffer counter ions and sucrose.

The (b) (4) and excipients (sucrose and salts) are controlled to maintain solubility and stability of the active ingredients and are suitable for intravenous infusion.

Additional characterization studies

(b) (4)

Table 4: Principle of physicochemical methods.

(b) (4)

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

(b) (4)

2.2. Biological properties

FACTOR X contains human FX. This zymogen can be converted to activated FX to provide hemostatic potential. It represents the FX molecular species found in the healthy normal population because a single lot of FACTOR X is derived from plasma which is pooled from between (b) (4) plasma donations.

After reconstitution, the functional activity of the FX active ingredient is 100 IU/mL. Intravenous infusion of a small volume of FACTOR X in a patient with FX deficiency is expected to provide sufficient elevation to restore normal hemostasis.

In vitro and *in vivo* studies indicate that FACTOR X does not promote a thrombotic response and the product appears to be non-thrombogenic when assessed by routine clinical laboratory tests and non-routine characterization studies. In the absence of deliberate activators, FACTOR X does not demonstrate substantive proteolytic activity.

The protein composition and characterization of FACTOR X is further described below.

2.2.1. Factor X Assays

The potency and clinical dosing regimen of the FACTOR X product are defined in terms of International Units of human FX. These are defined by a World Health Organization International Standard preparation.

Factor X activity can be measured by its ability to clot plasma (clotting methods) and by its proteolytic ability to cleave a synthetic peptide substrate with consequent release of a chromophore (chromogenic methods). The FACTOR X final product potency is assigned using the chromogenic activity assay in accordance with the current (b) (4) method. There is no equivalent method specified in the current (b) (4)

The FX protein can also be measured immunologically in terms of FX antigen, by determination of FX binding to an anti-FX antibody. These methods do not necessarily differentiate between active and non-active FX protein as long as the antigen epitope can be recognized by the antibody.

As methodology may vary in clinics at the point of use, the equivalence of the different methods has been evaluated. This section describes studies which have compared the results when these assays were performed on samples of FACTOR X final product and on human patient samples analyzed during the clinical trials.

Comparison of Assays using FACTOR X Final Product

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

(b) (4)

(b) (4)

Comparison of Assays using human plasma samples

At FDA's request, BPL conducted analysis of the differences between the chromogenic and clotting methods of FX determination in patient samples. FX activity was measured at the central testing

laboratory (b) (4)) using clotting and chromogenic assay methods during the clinical trial Ten01. A Z-test of significance was used because the sample was greater than or equal to 30. The criteria for this test are:

- if $-1.96 < Z < 1.96$, then the null hypothesis is not rejected, i.e., there is no difference in the means;
- if $Z > 1.96$ or $Z < -1.96$, then the null hypothesis is rejected and there is a significant difference in the two populations.

The pooled results for the patient samples are summarized in the Table 9 below.

Table 9: Summary of the pooled data for FACTOR X TEN01 pharmacokinetics samples tested by central laboratory	Factor X Chromogenic, IU/mL		Factor X Clotting, IU/mL
	n	119	119
	Average	0.31655462	0.267143
	SD	0.23478962	0.206858
	Variance	0.05512616	0.04279
	Z		Outcome
	Chromogenic versus clotting	-1.72	Accept null hypothesis: no difference in data

Conclusions

The data from (b) (4) on the patient pharmacokinetic samples show that there is statistically no difference between the factor X chromogenic assay and the clotting assay.

Reviewer comment: *I found that the chromogenic assay gives slightly higher FX values in the PK curves obtained with the chromogenic and clotting assays, see overlay Figure 4 below.*

1 page determined to be not releasable: (b)(4)

(b) (4)

Overall Conclusion of Factor X Assay Studies

BPL concludes that there was no statistically significant difference between factor X clotting assays and factor X chromogenic assays, when used for measurement of factor X in FACTOR X final product or in clinical plasma samples. ***Reviewer comment: I think that additional statistical analyses of the potency ratios are needed to support this conclusion.***

BPL proposes that conducted studies justify the use of the chromogenic assay for labelling and release of FACTOR X final product. ***Reviewer comment: From the assay perspective, I agree with BPL. However, since most clinical laboratories use the clotting assay, therefore we should advise BPL to label the FX product by the clotting assay.***

There was a statistically significant difference between the activity and antigen assays. This may indicate the presence of some non-functional protein in the FACTOR X final product, but more

probably is an artefact of the uncharacterized factor X protein integrity in the assay standard.

Reviewer comment: *I agree.*

2.2.2. Analysis of plasma protein impurities (FACTOR X protein composition)

As part of the characterization of the product, FACTOR X has been tested for the presence of other plasma protein impurities. These were identified from previous studies which analyzed the composition of manufacturing intermediates isolated from the pooled plasma and (b) (4)

Determination of coagulation factor impurities using specific assays

Materials and Methods

Table 12 below summarizes the analytical method used for each of the proteins. The assays used for FII and FIX are the FACTOR X final product release assays. (b) (4) and (b) (4) were measured using an (b) (4) method. Except for the (b) (4) assay, all methods were shown to be fit for purpose prior to testing of the FACTOR X batches.

(b) (4)

(b) (4)

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

(b) (4)

2.2.3. Activated coagulation factors

The non-activated partial thromboplastin time (NAPTT) is a chronometric test consisting of (b) (4)

. Samples containing activated clotting factors of sufficient concentration will shorten the clotting time. There is a (b) (4)

The test is not standardized – (b) (4)

FACTOR X final product is routinely tested for NAPTT. FACTOR X batches are characterized in passing the NAPTT test with long clotting times at the (b) (4), see figure below.

(b) (4)

The purpose of this study was to investigate the possibility of inhibition by the product and the sensitivity of the NAPTT to low concentrations of activated factor X (FXa). The sensitivity to FXa was selected because it is the most likely the activation product which could be generated from the predominant FX protein in FACTOR X.

(b) (4)

(b) (4)

Reviewer comment: *These data demonstrated the suitability of NAPTT for the detection of FXa impurity. Furthermore, the approach of release testing of samples at (b) (4), and setting a specification limit of (b) (4) are justified.*

3. Manufacturing process

3.1. Manufacturer(s)

Manufacture and packaging of FACTOR X is performed at a single location:

- Bio Products Laboratory Limited (BPL), Dagger Lane, Elstree, Hertfordshire, WD6 3BX, United Kingdom

Testing Premises

The majority of FACTOR X analytical testing is performed at the BPL manufacturing site:

- BPL, Dagger Lane, Elstree, Hertfordshire, WD6 3BX, United Kingdom

Additional contract quality control testing is performed at other locations as shown below:

- Nucleic Acid Amplification Testing (NAT) of the plasma batch fractionation pool for viral nucleic acid is performed at:
 - (b) (4)
- Sterility testing is performed either at BPL or at the following approved test facilities:
 - (b) (4)
- General Safety testing is performed at the following approved test facility:
 - (b) (4)

Plasma pool testing facilities are described below.

3.2. Batch Formula

(b) (4)

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

3.4. Source material (plasma)

The source material for FX manufacture is human plasma collected in the USA. **Reviewer note:** *BPL has incorrectly listed plasma as a Drug Substance (DS, section 3.2).*

BPL has established the Manufacturers Contractual Arrangement (MCA) with (b) (4). The Specification and Technical Agreement for Plasma from (b) (4) (signed in 2010) was submitted in the original BLA. The MCA sets out the following minimum requirements for plasma to be supplied to BPL sourced from the USA:

1. (b) (4)

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

(b) (4)

(b) (4)


3.6. FACTOR X Process Description

The FACTOR X manufacturing process is described below. The steps are identified by letter codes which are shown in the flow chart above. Steps (b) (4) are part of the long-established BPL manufacturing fractionation process for plasma. Steps (b) (4) are specific to the manufacture of FACTOR X.

(b) (4)

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


(b) (4)



3.6.8. Aseptic Filling and Freeze-Drying

Factor X is dosed into vials to achieve the target final dose size, then freeze-dried for long-term stability.




(b) (4)



3.6.9. Terminal Dry Heat-Treatment

The product undergoes terminal dry heat treatment in the closed final container, to inactivate viruses.


(b) (4)



3.8. Reagent solutions and buffers

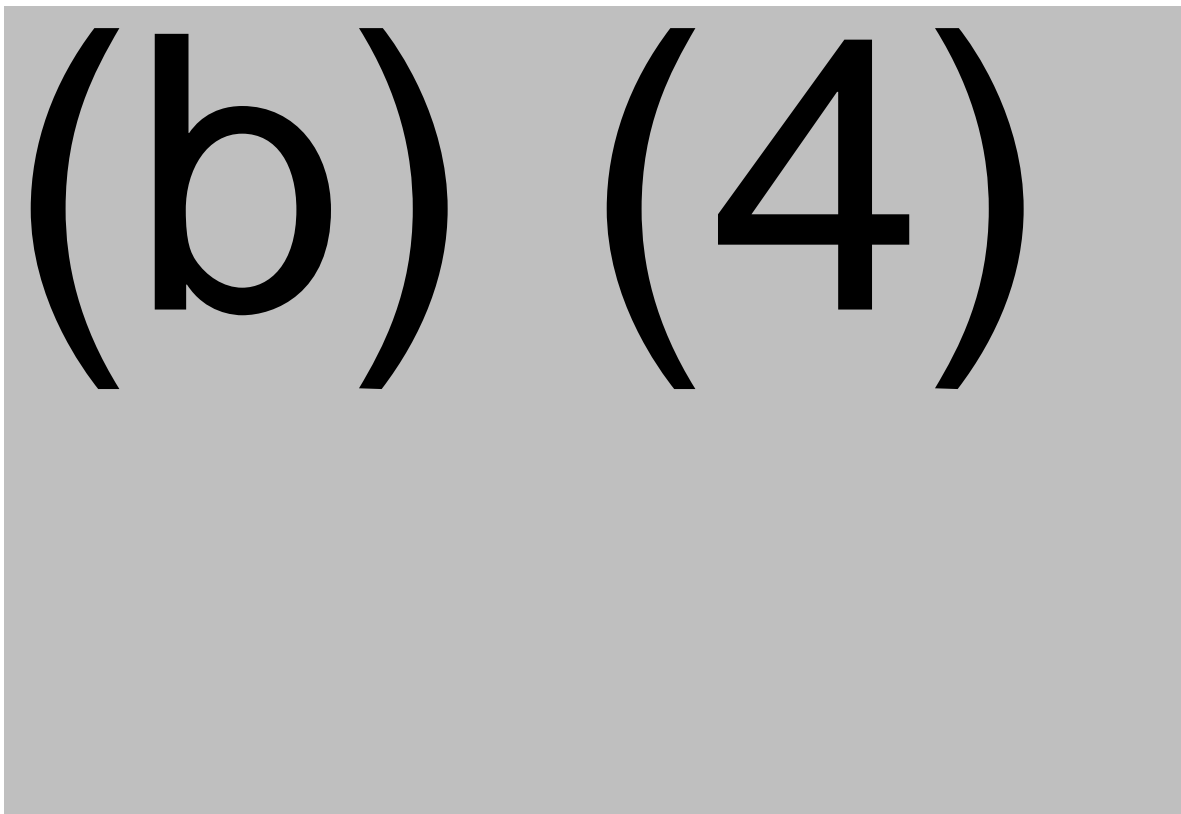



Operational tolerances on component concentrations are determined by weighing capability, and controlled by compliance with the required solution properties (b) (4)

(b) (4)



1 page determined to be not releasable: (b)(4)

•(b) (4)



4. Pharmaceutical Development

4.1. *Formulation development*

Final drug product presentation

The formulation of FACTOR X was optimized experimentally to provide physical and functional stability of the product during sterilizing filtration, freeze-drying, heat treatment and storage, and to ensure rapid resolution of the freeze-dried material.

The formulation of FACTOR X has been the same for all pre-clinical, clinical and stability studies.

FACTOR X is a freeze-dried powder which is reconstituted in Sterilized Water for Injections (sFWI) (b) (4) solvent prior to use. **Reviewer comment:** *The BLA contains multiple references to the (b) (4). BPL has been requested to comply with the (b) (4) where such monographs are available.*

FACTOR X is presented in a glass vial containing 250 IU or 500 IU of FX per vial. Dose size is determined only by the weight of product filled into the vial. Primary packaging (vial, stopper and over-seal) is the same for both dose sizes. After reconstitution, both dose sizes contain the same concentration of drug product and excipients. For this reason, individual pre-clinical or clinical studies using one of the two dose sizes are representative of both dose sizes. Stability studies have included both dose presentations. The product formulation is described below (Table 23). Factor X protein is compatible with all excipients at the concentrations used, as demonstrated by stability studies carried out on the product both in the freeze-dried state and after reconstitution.

Table 23: Product formulation.

Ingredient	Concentration	Function
Factor X	100 IU/mL nominal; (b) (4)	Active ingredient. Provides hemostatic control by replacement in factor X-deficient patients.
Protein	(b) (4)	Predominantly factor X
Citrate ((b) (4))		(b) (4)
Phosphate ((b) (4))		
Chloride ((b) (4))		
Sodium ((b) (4))		
Sucrose	(b) (4)	Stabilizes factor X across freeze-drying, heat-treatment and storage.

NLT, not less than

NGT, not greater than

FACTOR X vials are (b) (4) glass ((b) (4) Stoppers are of (b) (4) rubber. Over-seals consist of a clear lacquered aluminum skirt with a “flip-off” cap, providing a tamper evident seal when crimped on to the glass vial.

Diluent vials are (b) (4) glass with a (b) (4) rubber stopper.

Before formulation, the functional activity of FX may vary, but the concentration of inorganic salts and sucrose will be approximately constant. Potency Adjustment Buffer contains the same concentration of these excipients, so dilution of this intermediate to achieve the target FX potency does not substantively alter the excipient concentrations.

Formulation Development

The formulation of FACTOR X has been derived from the composition of elution buffers used during (b) (4) purification. During development of those (b) (4) steps, citrate and phosphate salts were used to (b) (4)

These salts are accepted pharmaceutical excipients and have been well-tolerated in other plasma-derived products.

4.2. Manufacturing Process Development

The manufacturing process for FACTOR X was designed to be compatible with an existing fractionation process which also extracts other therapeutic proteins from a single plasma pool.

Coagulation Factor X is one of the so-called “Vitamin K-dependent” proteins in the prothrombin complex (Factors II, VII, IX and X), which share common chemistry in partial amino acid sequence homology and post-translational carboxylation of glutamic acid residues. For this reason, they co-purify in many common processes associated with the fractionation of plasma.

The purification of FX therefore utilizes initial steps which are established parts of the BPL plasma fractionation process flow. These established parts yield a partially-purified FX concentrate. Subsequent steps dedicated to the final purification of FX have been developed to achieve the quality target product profile for FACTOR X.

The following description of pharmaceutical development therefore includes historical information which defined those parts of the process which were already established before development of the FACTOR X product, and contemporaneous information to confirm these steps for FACTOR X manufacture and to define the dedicated FACTOR X process steps.

(b) (4)

(b) (4)

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

4.3. Container/Closure System Development

The 250 IU and 500 IU FACTOR X dose sizes are both presented in (b) (4) glass 10 mL vials with a 20 mm neck. Each is stoppered with a 20 mm (b) (4) freeze-drying stopper and sealed with a 20 mm crimp-on over-seal with a flip-off (tamper-evident) center. The closure system protects FACTOR X from exposure to moisture by sealing the product under vacuum on completion of the freeze-drying process. A needle-free transfer device (Mix2Vial™, US 510(k) number K031861) is supplied with the product, facilitating transfer of water into the FACTOR X vial and of reconstituted product out of the vial and into the administration device (not supplied).

BPL has evaluated the container closure integrity in several different ways:

- (b) (4) of the closure system in (b) (4) ;
- (b) (4)
- Stability study of FACTOR X product sterility at end of shelf-life;
- (b) (4) testing by (b) (4) in FACTOR X samples at the end of shelf-life;
- (b) (4) testing of all FACTOR X batch vials, using (b) (4)

All these studies are supportive of the container closure system integrity for the duration of FACTOR X shelf-life. Two additional studies were conducted which supported the pharmaceutical development of the FACTOR X container closure system. The (b) (4) in the closure system was evaluated by a stability study over 36 months; and the stability of freeze-dried FX protein was evaluated after repeated exposure to (b) (4) testing.

4.4. Microbiological Attributes

The FACTOR X manufacturing process contains several steps which contribute to the microbiological safety of the finished product. These steps include three dedicated virus clearance steps (solvent-detergent treatment, virus filtration, and terminal dry heat treatment), as well as (b) (4) procedures which reduce the bioburden in the process solution (b) (4). Prior to filling into the final container, the product is filtered through a (b) (4) sterilizing-grade filter, which has been validated for bacterial reduction capability. (b) (4). The manufacturing environment, utilities and equipment are designed and operated to control bioburden on the product.

Reviewer comment: *The validity of the claims regarding equipment cleaning and sterility of manufacturing environment have been reviewed by Dr. Randa Melhem, DMPQ. Dr. Melhem found deficiencies which were confirmed during the pre-approval inspection in November 2013. Several Information Requests regarding these deficiencies remain unanswered as of 21 February 2014.*

4.5. Compatibility of FX with buffers, equipment, containers, and injection device

The compatibility of FACTOR X, and its manufacturing process intermediates, has been considered with regard to formulation buffers, product-contact process equipment, final product container and transfer device, including the stainless steel material. During the pharmaceutical development process, scrutiny of functional activity, yield and potential thrombogenicity across process stages did not identify any incompatibility with the integrity or activity of the FX protein.

5. Process Validation and/or Evaluation

The FACTOR X manufacturing process comprises three previously established steps which yield a FX-enriched intermediate. The previously established steps have been part of the routine fractionation of plasma pools for the extraction and manufacture of plasma proteins **for more than 15 years**. They were introduced before the industry and regulators had developed formal strategies for process validation. The dedicated FACTOR X process steps have been operated at full-scale at the BPL GMP facility for the manufacture of clinical trial material since 2007.

Elements of Process Validation

Process Validation for FACTOR X follows the structure of Process Design, Process Qualification and Continued Process Verification, as described by the FDA “Guidance for Industry - Process Validation: General Principles and Practices”.

1. Process Design, based on the target product profile and quality attributes, is described as part of pharmaceutical development.
2. Process Qualification has been performed as follows:
 - a. Design of the manufacturing facility and qualification of the utilities and equipment;
 - b. Process performance qualification has been performed on the dedicated steps of FACTOR X manufacture.
3. Continued Process Verification is routinely performed on the established (b) (4) process steps, according to process- and product- monitoring procedures. In the absence of a prospective process performance qualification at the introduction of these steps, the accumulated process data of many batches over many years has also been evaluated by a retrospective statistical process qualification. Although fewer in number, the dedicated FACTOR X batches have also been evaluated using the same statistical tools.
4. Specific validation of critical process steps was performed for the (b) (4) (reviewed by Dr. Randa Melhem, deficiencies were found).
5. The validation of intermediate storage conditions was conducted for all intermediates.
6. The validation of (b) (4) is reported below.
7. Container Closure has been validated.
8. The validation of equipment cleaning was reviewed by Dr. Randa Melhem.
9. The validation of transport of the drug product is consistent with transportation conditions for other plasma-derived products at BPL.

Results of the continued process validation are presented in Table 30.

Table 30: Results of Continued Process Verification for the Established Steps

Process step	Study design	Results
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(b) (4)

5.1. Process validation of dedicated FACTOR X manufacturing steps

Formal process validation has been performed in 2008-2009 according to the process validation protocol. The conformance batches of FACTOR X have been manufactured at commercial scale for the provision of material to the clinical trial at the early stages of the clinical program. FACTOR X was manufactured from established intermediates generated during the routine plasma fractionation stream.

Three consecutive conformance batches of FACTOR X were included in the execution of this protocol (Table 31).

Table 31: Conformance batches. Intermediate Batch	Date of start of Manufacturing	Finished Product Batch number	Date of fill	Fill size	Freeze- dryer Plant number

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

(b) (4)

5.6. Validation of container closure system

Container closure integrity has been demonstrated for this FACTOR X closure by studies which tested (b) (4)

These studies have been reviewed by Dr. Randa Melhem, DMPQ.

6. Control of Drug Product

6.1. Analytical Procedures

The analytical procedures for testing the FACTOR X drug product are summarized in Table 36.

Reviewer comment: *In general, the list of analytical procedures developed for evaluation of the FACTOR X product is adequate for the characterization of a plasma-derived coagulation factor concentrate.*

Table 36: Analytical Procedures for FACTOR X

Analytical Test	Method	Compliance [a]	Pharmacopoeial principle
Characteristics			
Appearance of freeze-dried plug	Visual inspection	BPL	(b) (4)
Residual water content	(b) (4)	BPL	
Solubility	Visual inspection / chronometer	BPL	
Appearance of solution	Visual inspection	BPL	
(b) (4)	(b) (4)	BPL	
(b) (4)	(b) (4)	BPL	
Stability	Visual inspection	BPL	
Identity	Assay	BPL	
Biological Safety Tests			
Sterility	Membrane filtration	BPL	
Endotoxin	(b) (4)	BPL	
General Safety Test	Animal test	BPL	CFR
Purity/Specific Function			
Factor X	Chromogenic	BPL	(b) (4)
Total Protein	(b) (4)	BPL	
Specific activity	Calculation	BPL	
NAPTT	Clotting time	BPL	
FCT	Clotting time	BPL	
Excipients			
Chloride	(b) (4)	BPL	
Phosphate	(b) (4)	BPL	
Citrate	(b) (4)	BPL	
Sucrose	(b) (4)	BPL	
Sodium	(b) (4)	BPL	
Impurities			
Factor II	(b) (4)	BPL	
Factor IX	(b) (4)	BPL	
(b) (4)	(b) (4)	BPL	
(b) (4)	(b) (4)	BPL	
(b) (4)	(b) (4)	BPL	

[a] There are no pharmacopoeial monographs for human coagulation factor X. Compliance standards have been set by Bio Products Laboratory (BPL).

As an example, description of the potency assay is provided below.

Determination of Factor X (potency assay)

The method follows the current edition of the (b) (4) method for the assay of human coagulation FX. The activity of FX is determined by (b) (4)

FX activity is determined using (b) (4) human, or an equivalent standard calibrated in International Units against the current WHO International Standard for Factors II and X Concentrate.

Reviewer comment: *All currently used FX activity standards should be listed in the BLA. Introduction of new or alternative FX standards should be reported to the FDA.*

Assay Procedure

(b) (4)

(b) (4)

6.2. Validation of analytical procedures

The validation of analytical methods have been reviewed by the laboratory of Dr. Lokesh Bhattacharyya (DBSQC/ OCBQ) and Dr. Andrey Sarafanov (LH).

The following methods were found to be adequately validated:

- *Determination of Chloride*
- *Determination of Phosphate*
- *Determination of (b) (4)*
- *Determination of (b) (4)*
- *Characteristics, solubility, appearance of reconstituted solution*

For some assays, validation was attempted but failed. An example of commutation with the firm regarding failed validation of Determination of Factor X by chromogenic assay is presented below:

Information Request submitted on 9 October 2013:

3. Validation Report (3.2.P.5.3) for Determination of Factor X

- a. *You have not studied specificity of this assay citing that it is a (b) (4) procedure. You need to perform specificity study to demonstrate that the method works for your product without*

interference from the product matrix. Please provide data to demonstrate specificity of this assay based on analysis of representative product samples and matrices.

- b. You have demonstrated accuracy of the method by testing one standard (3rd International Standard) against another standard ((b) (4)). Please provide results of accuracy of your method using your product for which this assay is intended. We suggest you evaluate accuracy using spike-recovery method in which unspiked samples at different concentrations and the same samples after spiking with known concentrations (IU/mL) of the standard are analyzed.

BPL's Preliminary Response on 18 October 2013:

Specificity was not determined as the assay used both the specific Factor X activator, (b) (4) and the chromogenic substrate for activated Factor X as described in the (b) (4) method, hence the assay was deemed to be specific for Factor X. However as specificity was not determined using representative product samples, validation will be carried out with final product.

BPL's Final Response on 31 January 2014:

Validation protocol LP/403/1/23/01 and interim report LR/403/1/23/01 are presented in **Appendix II**. Accuracy did not meet its acceptance criteria and is being investigated under QR77474. A report for the completed validation will be supplied by the 14th March 2014.

As of 24 February 2014, validation of at least one parameter for the following methods has failed:

1. Potency assay *Determination of Factor X (Chromogenic Assay)*,
2. Thrombogenic impurity assays *Determination of Non-Activated Partial Thromboplastin Time (NaPTT)* and *Determination of Fibrinogen Clotting Time (FCT)*,
3. Coagulation factor impurity assays *Determination of Factor II* ((b) (4) Assay) and *Determination of Factor IX* ((b) (4) Assay), and
4. Solvent-detergent impurities assay *Determination of* (b) (4) .

Insufficient validation was identified for the following methods:

1. (b) (4) *Moisture Determination*,
2. *Determination of Total Protein* by (b) (4)
3. *Determination of Citrate*,
4. *Determination of* (b) (4)
5. *Sucrose Determination* by (b) (4) and
6. *Determination of Sodium Content* by (b) (4) .

6.3. Batch Analyses

A total of (b) (4) FDP batches are reported in the BLA (Table 37). A subset of these batches has been included in stability studies to establish product shelf-life.

(b) (4)

Reviewer comment: Analysis of 250 IU and 500 IU FDP batches, presented in Tables 38 and 39 below, demonstrates consistent potency and impurity levels of the released commercial-scale batches. Please note that some of the manufactured batches were rejected and are not included in this table (see section 5. Process Validation and/or Evaluation)

Table 38: FACTOR X 250 IU Drug Product Analysis: Commercial-Scale Batches

(b) (4)

1 page determined to be not releasable: (b)(4)

(b) (4)

6.4. Characterization of Impurities

The impurities in FACTOR X can be grouped into three classifications:

1. Plasma proteins impurities in the (b) (4) which are co-purified throughout the manufacturing process.
2. Non-protein impurities which are introduced during the manufacturing process and may not be completely removed in the drug product.
3. (b) (4)

6.4.1. Plasma Protein Impurities

Batches of FACTOR X which were representative of the 250 IU and 500 IU dose sizes were analyzed for plasma protein impurities in a non-routine characterization study described above. Factor II (prothrombin), FIX, (b) (4) of the total protein in FACTOR X. Other proteins were detected in smaller, trace amounts (b) (4). The concentration of all identified protein impurities was below the reported concentrations of those proteins in normal plasma when calculated per 100 IU of the main pharmaceutical ingredient FX, see Table 40.

Table 40: Major Plasma Protein Impurities in FACTOR X

Protein	Mean Concentration, µg/mL	Number of batches tested	Range, µg/mL	Normal plasma concentration, µg/mL	% of total FACTOR X protein
Factor II	(b) (4)				
Factor IX					
(b) (4)					
(b) (4)					

[a] ND, not detectable in 7 of (b) (4) batches. Mean concentration was calculated from quantified values only

[b] Less than limit of quantitation in 2 of (b) (4) batches. Mean concentration was calculated assuming limit of quantitation value.

Factor II and Factor IX

Reduction of FII and FIX in FACTOR X is desirable, in order to:

- minimize the total protein load in each clinical dose;
- maximize the specificity of treatment; and
- minimize the thrombogenic potential contribution of prothrombin and FIX zymogens or their activated proteases.

For these reasons, FII and FIX are measured by specific assays in each batch of FACTOR X to demonstrate compliance with the drug product specification. The presence of any activated FII or FIX are measured in each batch by the global hemostasis tests for activated clotting factors (NAPTT) and thrombin (FCT).

(b) (4)

6.4.2. Non-Protein Impurities

Contact chemicals which are added during the FACTOR X manufacturing process are measured in the routine testing of final product against specification. These comprise excipients (salts and stabilizer) present within specified limits and impurities added and then substantially removed during the manufacturing process, as demonstrated by Table 41 below.

Table 41: Non-Protein Impurities in FACTOR X

	Specification Limit	Mean Concentration	Number of batches tested	Batch Range
Excipients	(b) (4)	(4)		
Chloride, (b) (4)				
Phosphate, (b) (4)				
Citrate (b) (4)				
Sucrose, (b) (4)				
Sodium, (b) (4)				
Impurities				

(b) (4)

NGT, Not Greater Than

[a] Less than limit of quantitation. Mean concentration was calculated assuming limit of quantitation value.

(b) (4)

(b) (4)

6.5. Justification of Specifications

The specification for each test parameter is justified below.

Description of freeze-dried plug

FACTOR X is supplied as a freeze-dried material in a closed glass vial. This test confirms the visual characteristics of the product before reconstitution. Inspection of the product to confirm compliance with the description “a white powder of (b) (4)” has been adopted from the (b) (4) which is a comparable coagulation factor product to FACTOR X. The use of a qualitative description is also described in the (b) (4). The description has been defined by BPL to reflect the normal appearance of FACTOR X product.

Moisture

FACTOR X is supplied as a freeze-dried product. The stability of the product over shelf-life will be influenced by the moisture (also known as residual water content). Use of this test is derived from the (b) (4) which is a comparable coagulation factor product to FACTOR X. The test is also described in the (b) (4). The BPL limit for FACTOR X is (b) (4), based on previous experience with freeze-dried coagulation factor products.

The **solubility** time indicates integrity of the freeze-dried FACTOR X product. This product attribute is a reflection of non-routine tests to analyze (b) (4). The test is derived from the (b) (4) which is a comparable coagulation factor product to FACTOR X. The upper limit of (b) (4) has been defined by BPL to reflect current FACTOR X batch performance and allow for procedural differences at the point of use.

Appearance of solution: Inspection of the reconstituted product to confirm compliance with the description “colorless, clear or slightly opalescent solution” has been adopted from the (b) (4) which is a comparable coagulation factor product to FACTOR X. The use of a qualitative description is also described in the (b) (4). This product attribute is a reflection of non-routine tests to analyze (b) (4).

(b) (4)

Stability ensures that the product remains usable for a period of time after reconstitution. This product attribute is a reflection of non-routine tests to analyze (b) (4). The BPL limit applied for FACTOR X provides a (b) (4) margin of safety beyond the time which is specified for use in the FACTOR X prescribing information.

Identity: This BPL test is a positive confirmation of product identity, based on reactivity in the factor X assay. This test is derived from the (b) (4) which is a comparable coagulation factor product to FACTOR X. An identification test is also required by (b) (4).

Biological Safety Tests

Sterility Product compliance with sterility testing is a general requirement of (b) (4).

Endotoxin (b) (4) The test for gram-negative bacterial endotoxin detects most common cause of pyrogenic activity in pharmaceutical products.

General Safety Test: This animal safety test is required in compliance with (b) (4). FACTOR X batches must pass the general safety test. 21 CFR 610.11 also provides for an applicant to justify not performing the test. At the time of initial BLA filing, there are insufficient batch data to provide such justification; (b) (4).

Purity/Specific Function

Factor X activity is measured to assign the actual potency to each batch of FACTOR X and to confirm product identity. The limits are defined in accordance with the (b) (4) which is a comparable coagulation factor product to FACTOR X. T (b) (4) of the stated potency. As the FACTOR X stated potency is 100 IU/mL after reconstitution, the BPL limits on potency have been defined as 80 IU/mL to (b) (4) IU/mL.

The labelling of FACTOR X reports the total units of FX activity per vial. This protects against any variation in reconstitution procedure at the point of use. The limits on FX per vial reflect the (b) (4) limits on FX activity for each dose size. Thus the BPL limits for FX per vial are 200 IU – (b) (4) IU for the 250 IU dose (reconstituted in 2.5 mL) and 400 IU – (b) (4) IU for the 500 IU dose (reconstituted in 5 mL).

(b) (4)

Total Protein indicates the purity of FACTOR X in terms of other unspecified contaminating proteins (b) (4). The BPL upper limit for total protein in FACTOR X was defined by the (b) (4)

The **specific activity** of FX in FACTOR X drug product describes the purity in terms of other protein impurities (b) (4). Purity is proportional to specific activity, which is the ratio of FX functional activity: total protein. This product attribute is a reflection of non-routine tests to analyze (b) (4). Assignment of a lower limit for specific activity follows the principle set out in the (b) (4), which is a comparable coagulation factor product to FACTOR X. For that product, the minimum specific activity is (b) (4) and FX proteins are of similar size and circulate in plasma at similar molar and mass concentrations, so use of comparable limits for FACTOR X is justifiable. The BPL FACTOR X limit of 80 IU/mg of protein reflects a value which is three standard deviations from the mean of batches manufactured.

The **non-activated partial thromboplastin time (NAPTT)** is a measure of activated clotting factors in the product. The test is considered to be a measure of the potential thrombogenicity of coagulation factor products, particularly those which include zymogens which can be activated to coagulant proteases. The limits are defined in accordance with the (b) (4) which is a comparable coagulation factor product to FACTOR X. The NAPTT lower limit for FACTOR X is (b) (4), reflecting the (b) (4) limit. Results are reported as absolute clotting times in a test for which there is no standard. Therefore the blank NAPTT clotting time is also reported and must remain within limits set by the (b) (4). The

FACTOR X specification requires NAPTT to be reported as the measured clotting time for each batch.

The **fibrinogen clotting time (FCT)** measures low concentrations of thrombin. The test is considered to be a measure of the potential thrombogenicity of coagulation factor products, particularly those which include FII (prothrombin) zymogen which has the potential for activation to thrombin. The FCT test is described as a test for thrombin in (b) (4). BPL has retained the FCT test in the FACTOR X specification because FII is the main contaminating protein, although reduced by orders of magnitude compared to a PCC. FACTOR X is tested using the method performed at (b) (4), which best reflects the physiological environment during clinical use. Accordingly, the BPL lower limit clotting time of (b) (4) has been defined in accord with the (b) (4) limit for other products. This approximates to a thrombin concentration of (b) (4).

Excipients

Chloride provides (b) (4) the FACTOR X product. The presence of chloride in the product is due (b) (4). The specification provides a range which reflects the target concentration at formulation and the available data for batch performance rounded mean (b) (4) standard deviations ((b) (4)). The FACTOR X specification for chloride has been defined by BPL as (b) (4).

Phosphate provides (b) (4) to the FACTOR X product. The presence of phosphate in the product is due to (b) (4). The specification provides a range which reflects the target concentration at formulation and the available data for batch performance rounded mean (b) (4) standard deviations ((b) (4)). The FACTOR X specification for phosphate has been defined by BPL as (b) (4).

Citrate provides (b) (4) to the FACTOR X product. The presence of citrate in the product is due to (b) (4). The specification provides a range which reflects the target concentration at formulation and the available data for batch performance rounded mean (b) (4) standard deviations ((b) (4)). The FACTOR X specification for citrate has been defined by BPL as (b) (4).

Sucrose provides stabilization for FX protein in the FACTOR X product. The addition of sucrose to the FACTOR X formulation is controlled during manufacture. The specification provides a range which reflects the target concentration at formulation and the available data for batch performance rounded mean (b) (4) standard deviations ((b) (4)). The FACTOR X specification for sucrose has been defined as (b) (4).

Sodium is the (b) (4) in the FACTOR X product. It is introduced into the product (b) (4). The specification provides a range which reflects the target concentration at formulation and the available data for batch performance rounded mean (b) (4) standard deviations ((b) (4)). The FACTOR X specification for sodium has been defined by BPL as (b) (4).

Impurities

Factor II (prothrombin) is the main protein impurity in FACTOR X. It is substantially reduced by control of the (b) (4) steps during FACTOR X manufacture. The specification limit for factor II in FACTOR X confirms this reduction. It also ensures that infused FACTOR X will not elevate the circulating plasma concentration of FII beyond normal (1 IU/mL). This value approximates available batch performance data for the mean (b) (4) standard deviations ((b) (4)).

Factor IX is the other coagulation factor protein impurity in FACTOR X. It is substantially reduced by control of the (b) (4) steps during FACTOR X manufacture. The specification limit for FIX in FACTOR X confirms this reduction. It also ensures that infused FACTOR X will not elevate the circulating plasma concentration of FIX beyond normal (1 IU/mL). This value approximates available batch performance data for the mean (b) (4) standard deviations ((b) (4)).

(b) (4)

(b) (4)

(b) (4)

6.6. Reference Standards or Materials

Factor X Standard

Factor X activity in FACTOR X intermediates and final product is measured against a FX standard preparation. This is either the WHO International Standard for Factors II and X Concentrate,

(currently the 3rd International Standard for Factors II and X Concentrate, 98/590) or a local standard which has been calibrated against the International Standard.

Reviewer comment: *All currently used FX activity standards should be listed in the BLA. Introduction of new or alternative FX standards should be reported to the FDA.*

Protein Standard Protein concentration in FACTOR X intermediates and final product is measured against a protein standard prepared from (b) (4).

Non-activated Partial Thromboplastin Time Control. NAPTT measures the absolute clotting time of a treated sample. There is no standard for this test. However, each test includes a positive control sample which has been prepared from a prothrombin complex concentrate process intermediate and demonstrates a NAPTT within a range close to the test limit ((b) (4)).

Fibrinogen Clotting Time Control. FCT measures the absolute clotting time of a sample. There is no standard for this test. However, each test includes a positive control sample of thrombin which, when diluted to specified concentrations, demonstrates the expected clotting times across the measured range (approximately (b) (4)).

Factor II activity in FACTOR X intermediates and final product is measured against a **FII standard preparation**. This is either the WHO International Standard for Factors II and X Concentrate, (currently the 3rd International Standard for Factors II and X Concentrate, 98/590) or a local standard which has been calibrated against the International Standard.

Factor IX activity in FACTOR X intermediates and final product is measured against a FIX standard preparation. This is either the WHO International Standard for Blood Coagulation Factor IX Concentrate, (currently the 4th International Standard for Blood Coagulation Factor IX Concentrate, 07/182) or a local standard which has been calibrated against the International or National Standard.

7. Stability Summary and Conclusions

Stability indicating parameters

The parameters chosen to profile the stability characteristics of FACTOR X are in accordance with the recommendations of ICH Topic Q5C, Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products. The principal stability indicating parameters are a subset of the FDP release assays: FACTOR X potency, assessment of appearance, solubility and sterility (sterility is tested at the end of each stability trial). Other methods selected from the finished product specification that are not necessarily stability indicating, have been included to demonstrate compliance with specification over the duration of the trial. The test for Identity is performed at Time Zero. The General Safety test has been performed at Time Zero for the last three batches. Neither of these tests are repeated during the course of the trial, as their status in a secure container closure system will not change. Also, as identity is confirmed by the detection of human FX within the required range in the potency assay, it can be concluded that the batches will still comply at 36

months. **Reviewer comment:** *As described above, not all tests are performed at all time-points. I agree that General Safety as well as Identity tests are not indicative of storage stability. The quality indicative parameters, Description of freeze-dried plug, Solubility, Appearance of solution, Stability, Factor X activity, Specific Activity, NAPTT (b) (4) APTT control, and FCT, were conducted at each storage time point, which appears to be acceptable.*

The storage temperatures are:

- (b) (4) (for use as a reference when estimating biological activity)
- +5°C (b) (4) (recommended / real-time storage)
- +25°C (b) (4) (recommended / real-time storage)
- +30°C (b) (4) (recommended / real-time storage)
- (b) (4) accelerated storage.

Results

All stability batches reflected commercial-scale manufacturing process (see Table 43) and met the FDP Specification limits at both +5°C, +25°C and +30°C storage conditions for the time-points reached. This includes (b) (4) batches (b) (4) batches at 250 IU and (b) (4) batches at 500 IU) which have reached proposed shelf life of 36 months. Analysis of Factor X potency confirms that potency is highly stable at +5°C, +25°C and +30°C, and that there is no significant difference between the two presentations. Similar profiles are seen for other tests parameters between both presentations. All data support the shelf-life claim of 36 months at +2°C to +30°C for 250 IU and 500 IU presentations of FACTOR X, within its original packaging, stored in the dark.

Table 43: Summary of Batches on stability

(b) (4)

[a] batches also used to test compatibility with the reconstitution device and stability of the reconstituted solution.

[b] (b) (4) .

Stability after reconstitution

(b) (4) manufacturing batches showed no decline in FX potency when reconstituted material was sampled after 1 hour at 25°C (b) (4) whether held upright or inverted in contact with the stopper. All data support the recommendation to use FACTOR X within 1 hour of reconstitution.

Post-approval Stability Commitment

The stability studies for the batches (b) (4) and (b) (4) will be continued for up to 36 months. BPL will inform the FDA if the stability studies show any deviation from specification.

8. Complete Response Letter

The following comments from the three discipline reviewers who found significant deficiencies with the analytical methods (Dr. Lokesh Bhattacharyya), process validation (myself), and facilities (Dr. Randa Melheim) need to be included the Complete Response letter. The CMC comments are arranged in the order of significance.

CMC, Analytical methods

1. The data you provided have not demonstrated that the following analytical methods used for the evaluation of potency and safety indicating parameters in the Final Drug Product (FDP) are adequately validated:
 - a. In the determination of *Factor X potency*,
 - i. Please revise SOP QCA/00179 to clearly state the assay validity (acceptance) criteria for the standard.
 - ii. Please describe clearly the details of the testing and calculation of potency in your SOP QCA/00089.
 - iii. Please provide data to demonstrate the *specificity* of this assay based on the analysis of representative product samples and matrices.
 - iv. Please provide results to support the *accuracy* of your method using your process intermediates and the FDP for which this assay is intended. We suggest you evaluate *accuracy* using a spike-recovery method in which you analyze non-spiked samples at different concentrations and the same samples after spiking with known concentrations (IU/mL) of the standard.
 - v. Please evaluate *linearity* at different dilutions of the product (dilution linearity) and show that the linear regression line of the standard and that of the product are parallel within the proposed assay range to validate that interpolation from the standard regression line is appropriate for the determination of the potency of the product.
 - vi. Please provide data to establish the *range* of the assay based on your results of repeatability, accuracy and linearity studies obtained using representative process intermediate and product samples over the intended range of the assay.
 - vii. Please provide data to demonstrate appropriate *robustness* of the assay method using representative process intermediate and product samples for which this assay is intended. The data should demonstrate the effect of small deliberate changes of critical method parameters, such as reagent concentration, incubation time, etc., as applicable.

- b. In the determination of *Total Protein* by (b) (4)
- Please provide data to support the *linearity* of the method using representative FDP samples, and to demonstrate *parallelism* between the linear regression fits for the FDP samples and the standard protein used in the linearity study.
 - Please provide data to establish the *range* of the assay based on your results of repeatability, accuracy and linearity studies obtained using representative product samples over the intended *range* of the assay.
 - Please note that the composition of your Internal Quality Control (IQC) is significantly different from that of the product, e.g., the average protein concentration of IQC is (b) (4) whereas the specification limit for the FACTOR X product is (b) (4). As a result, the IQC sample is not representative of the FDP, and it is not likely that any variation in the method will have similar effect on both FDP and IQC. Therefore, please provide data to demonstrate the *robustness* of your method in studies performed with representative FDP samples.
- c. In the determination of *Moisture* in Freeze-Dried Products by the (b) (4) Method,
- Please demonstrate method *specificity* using representative product samples.
 - Please provide validation data using representative product samples over the intended *range* of the assay. The following characteristics should be addressed: *specificity*, *accuracy* (spike recovery), *repeatability*, *intermediate precision* (multiple analysts, multiple days), *linearity*, *range*, *limit of quantitation (LOQ)* and *robustness* of the assay. We suggest that you spike your sample with different known amounts of water and then assay both non-spiked and spiked samples to calculate recovery.
- d. In the (b) (4) Method for the Determination of *Factor II* activity ((b) (4) Assay),
- Please provide data to demonstrate the *specificity* of this assay based on the analysis of representative product samples.
 - Please provide results to demonstrate method *accuracy* using FACTOR X product samples. We suggest you evaluate *accuracy* using a spike-recovery method by analyzing non-spiked samples at different concentrations and the same samples after spiking with known concentrations (IU/mL) of the standard in such a way that the total concentrations of Factor II in the samples are between the LOQ of the assay and the proposed specification limit.
 - Please provide data to assess the *LOQ* from analysis of representative samples of your product.
 - Please evaluate *linearity* at different dilution of the product (dilution linearity) and demonstrate that the linear regression line of the standard and that of the Factor II in your product are parallel within the proposed assay range to validate that interpolation from the standard line is appropriate for the determination of Factor II content of the product.

- v. Please re-evaluate the *range* of the assay based on your results of *repeatability*, *accuracy* and *linearity* obtained using representative product samples.
 - vi. Please provide data to demonstrate appropriate *robustness* of the assay method using representative product samples. The data should demonstrate the effect of small deliberate changes of critical method parameters, such as (b) (4), etc.
- e. In the determination of *Factor IX activity* ((b) (4) Assay),
- i. Please submit data to demonstrate the *specificity* of the assay by analyzing representative Factor X product samples to show that the results on Factor IX activities are not affected by the matrix at the concentration at which they are expected to be present in the product.
 - ii. Please evaluate *accuracy* using representative product samples. We suggest you evaluate *accuracy* using a spike-recovery method by analyzing non-spiked samples at different concentrations and the same samples after spiking with known concentrations (IU/mL) of the standard in such a way that the total concentrations of Factor IX in the samples are between the LOQ and the proposed specification limit for the product.
 - iii. Please provide data to determine the *LOQ* from the analysis of representative samples of your product for which the assay is intended.
 - iv. Please provide data, including your linear regression plots, to demonstrate *parallelism* between the linear regression fits for the FDP samples and the standard at different Factor IX concentrations.
 - v. Please re-assess the *range* of the assay based on your results of *repeatability*, *accuracy* and *linearity* obtained using representative product samples.
 - vi. Please provide data to demonstrate appropriate *robustness* of the assay method using representative product samples. The data should demonstrate the effect of small deliberate changes of critical method parameters, such as reagent concentration and incubation time, etc.
 - vii. Please provide the SOPs QCA/00042 and QCA/00073.
- f. In the determination of *Non-Activated Partial Thromboplastin Time* (NAPTT),
- i. Please validate NAPTT as a quantitative method with the actual time in seconds as the reportable result. In addition to *specificity*, please provide data to evaluate other validation characteristics appropriate for a quantitative test for impurity in terms of the reportable result.
 - ii. Based on our analysis of the calibration (qualification) data for the control you submitted, we found that the Mean^{(b) (4)} SD values are (b) (4) respectively. Please revise your SOP (QCA/00008) to include (b) (4) as the assay validity criteria.
 - iii. Regarding your response that “the operator will review the control chart and if the control result is not within^{(b) (4)} standard deviations of the control chart

mean, the assay would be considered invalid, and the results would not be used.”, please revise your SOP to include assay validity criteria.

- iv. Regarding your statement that (b) (4) are necessary to ensure that there is no masking, due to either over dilution or matrix inhibition, please include both dilutions as reportable results and revise your SOP (QCA/00008) accordingly.
- v. You indicated that (b) (4) step is not necessary for the Factor X product. Please revise your SOP (QCA/00008) to include this clarification.

g. In the determination of *Fibrinogen Clotting Time* (FCT),

- i. Please validate FCT as a quantitative method with the actual time as the reportable result. Please provide data to evaluate other applicable validation characteristics for a quantitative test for impurity in terms of the reportable result.
- ii. Please revise your SOP QCA/00011/15: The Fibrinogen Clotting Time Test to include appropriate and justifiable assay validity criteria.

h. In the determination of (b) (4)

i. (b) (4)

i. In the determination of (b) (4),

i. (b) (4)

(b) (4)

j. In the determination of *Sucrose* by (b) (4)

- i. Please provide data, including linear regression plots, to demonstrate *parallelism* between the linear regression fits for the FDP samples and the standard at different concentrations.
- ii. Please provide data to establish the *range* of the assay based on your results of *linearity*, *precision* and *accuracy* evaluation using representative samples of FDP.

k. In the determination of *Citrate* by (b) (4)

- i. Please evaluate *accuracy*, *repeatability* and *intermediate precision* over the actual assay range of (b) (4)
- ii. The *linearity* of the method was evaluated in the range (b) (4) however the *range* of the method was determined to be (b) (4) based on the *precision* and *accuracy* results, which is different than the range in which linearity was studied. Please provide additional data for the *linearity* over the stated *range* of the assay or re-define your assay *range* that is supported by *linearity*, *accuracy* and *precision* results.

l. In the determination of *Sodium* by (b) (4)

- i. Please provide data to show the *linearity* and *accuracy* of sodium response using FDP and *parallelism* between the standard and sample regression lines to demonstrate assay *linearity*.

m. In the determination of (b) (4)

(b) (4)

(b) (4)

CMC, process Validation

2. The data you provided do not adequately address the deficiencies in the validation of the FACTOR X manufacturing process that were identified during the Pre-license Inspection of your facility. Please provide data to demonstrate the following:
 - a. The execution of process validation protocol PV40300102 is able to result in FACTOR X batches that consistently meet pre-determined specifications.
 - b. The introduced manufacturing changes are able to correct the deficiencies of the manufacturing process.
3. Please establish specifications for all source materials per (b) (4), which should include, but not be limited to:
 - a. Release criteria for plasma pools, including Anti-HIV-1 & -2, HBsAg and Parvovirus B19-DNA
 - b. FACTOR X inactive ingredients, including sucrose, sodium (b) (4) and (b) (4) phosphate
 - a. Chemicals from the Manufacturing Batch Formula, including Citric acid (b) (4)
 - b. Sterile water for injection
 - c. Container closure system, including the glass vial
4. Please provide additional data to validate the following proposed manufacturing options:
 - a. With reference to FACTOR X Manufacturing Batch Formula (Table 3.2.P.3.2-T1), please provide data to validate the (b) (4)
 - b. Regarding the validation of the (b) (4) -filtration), please provide data to demonstrate the comparability in (b) (4)

- c. With reference to Section 3.2.P.3.3.1.2.8. *Step M: Aseptic filling and lyophilization*, please provide justifications for the following statement “*Excursions from these expected conditions would not result in batch failure, subject to compliance of the batch with final product specification after appropriate risk- and impact- assessment.*”
5. Please provide the protocol and qualification reports for the establishment of Factor X potency reference standards used for the release of FACTOR X.
6. Please address the following deficiencies regarding frozen plasma and plasma pools:
 - a. During the Pre-Licensure Inspection, you indicated that (b) (4) will not be used for FACTOR X manufacture. Please remove references to the use of (b) (4) from the BLA.
 - b. Please remove references to manufacturing steps and conditions that are not relevant to the manufacture of FACTOR X. For example, (b) (4) are used for the manufacture of (b) (4) products only.
 - c. (b) (4) plasma is considered as the source material for FACTOR X. Please transfer the information currently presented in Section 3.2. *Drug Substance* to Section 3.2.S.2.3 *Control of Materials*.
 - d. Regarding Plasma Container Closure System, you indicated that “Alternative containers when evaluated and approved will be accepted”. Please change this statement to “Alternative containers when evaluated and approved will be accepted and reported to the FDA”.
 - e. Please list all the facilities where plasma donations and plasma pools are tested in Section 3.2.S.2.1 *Manufacturers*.
7. Please address the following deficiencies regarding specifications:
 - a. (b) (4) Factor X (b) (4) intermediate prepared at the conclusion of *Step* (b) (4) (as indicated in the *Manufacturing Process Chart*) qualifies as the Bulk Drug Substance (BDS). Therefore,
 - i. Please list all manufacturing steps leading to this intermediate in Section 3.2. *Drug Substance*.
 - ii. Please develop BDS specifications, which can be comprised of existing parameters and acceptance limits for the intermediates “*Stabilized Factor X*” and “*Factor X eluate* ((b) (4))”.
 - iii. Please provide Batch Analyses for the BDS.
 - b. Please label FDP vials with the actual (not nominal) Factor X potency, and make the following changes to the FDP specification for “*Factor X activity per vial*”:
 - i. (b) (4) of nominal potency at release
 - ii. (b) (4) of labeled potency during the shelf-life of the product

- c. With reference to the deviation report 62654 related to the rejection of batch (b) (4) due to low potency caused by (b) (4), the associated change in (b) (4) was clearly demonstrated by the Factor X (b) (4). Therefore, please establish Factor X (b) (4) or (b) (4) as an additional identity and purity test for FDP. Please establish the specification as “comparable to a reference standard which is derived from FACTOR X”.
- d. Regarding the studies of the clotting and chromogenic assays for Factor X potency,
- i. For all pharmacokinetics (PK) and *in vitro* spiking studies, please evaluate the ratios of chromogenic to clotting potencies using statistical methods described in Bland JM and Altman DG, *Statistical methods for assessing agreement between two methods of clinical measurement*. *Lancet* 1986;1: 307-310
 - ii. For the *in vitro* spiking study presented in Table 3.2.P.2.2.3-T16, please explain the following:
 1. The test values from the parallel line clotting assay are noticeably smaller than those from the calibration curve-based clotting assay.
 2. Factor X potency values at release (labeled potency) derived from the clotting assays are noticeably less than those derived from the chromogenic assay.
 - iii. For the PK studies presented in Section 5.3.3 *Reports of Human PK Studies*, please explain why the chromogenic assay gives slightly higher Factor X potency values than the clotting assay, and comment on the potential implications for the safe and effective use of FACTOR X in clinical practice where the clotting assay is used predominantly in clinical laboratories.

FACILITIES & COMPLIANCE

8. Outstanding issues identified at the Pre-License Inspection performed on 12 - 25 October 2013 at the BPL facilities in Elstree, UK, and described in Form FDA 483 issued on 25 October 2013 have yet to be resolved. Please submit documentation that demonstrates that all outstanding inspectional issues identified during the PLI have been corrected.
9. You listed the minimum required time of the primary and secondary drying phases and the minimum duration of the heat treatment. Please provide the maximum allowed times for the drying phases and terminal heat treatment, and the studies performed to support these limits.