

CBER CMC BLA Review Memorandum

BLA STN 125671/0

**Antihemophilic Factor (Recombinant), GlycoPEGylated /exei
[ESPEROCT]**

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1. BLA#: STN 125671/0

2. APPLICANT NAME AND LICENSE NUMBER

Novo Nordisk, Inc.

3. PRODUCT NAME/PRODUCT TYPE

Non-Proprietary Name: Antihemophilic Factor (Recombinant), ClycoPEGylated / exei

International Non-Proprietary Name (INN): Turoctog alfa pegol

Proprietary Name (U.S. established): ESPEROCT

4. GENERAL DESCRIPTION OF THE FINAL PRODUCT

Pharmacological category: Antihemophilic Factor (Recombinant)

Dosage form: lyophilized powder for reconstitution

Strength/Potency: single-dose vials containing nominally 500, 1000, 1500, 2000 or 3000 International Units (IU) of factor VIII activity

Route of administration: intravenous (after reconstitution to solution)

Indication(s): for use in adults and children with hemophilia A for (i) on-demand treatment and control of bleeding episodes, (ii) perioperative management of bleeding and (iii) routine prophylaxis to reduce the frequency of bleeding episodes.

5. MAJOR MILESTONES

Submission Date – February 27, 2018

First Committee Meeting (internal) - March 3, 2018

Filing Meeting (internal) - April 13, 2018

Filing date – April 18, 2018

Mid-Cycle Meeting (internal) – July 27, 2018

Mid-Cycle Communication (external, teleconference) - August 08, 2018

Inspection of the manufacturing site (b) (4)

PeRC Meeting - September 19, 2018

Late-Cycle Meeting (internal) – November 2, 2018

Late-Cycle Meeting (external) – November 29, 2018

Labeling meetings – December 7, 2018 – January 18, 2019

Blood Products Advisory Committee meeting – waved

PDUFA action date – February 27, 2019

6. CMC/QUALITY REVIEW TEAM

Reviewer/Affiliation	Subject Matter (Section)
Andrey Sarafanov, PhD (<i>AS</i>); Office of Tissues and Advanced Therapies (OTAT)/ Division of Plasma Protein Therapeutics (DPPT)/ Hemostasis Branch (HB)	Drug Substance (DS) (except 3.2.S.4-7), Drug Product (DP) Powder (except sections 3.2.S.4-8) and DP Solvent. Selected sections of Module 3 were responsibility of Office of Compliance and Biologics Quality (OCBQ), Division of Manufacturing and Product Quality (DMPQ)
Alexey Khrenov, PhD (<i>AK</i>); OTAT/DPPT/HB	Control of DS and DP (3.2.S.4-5, 3.2.P.4-6, except potency control) & Analytical methods (5.3.1.4 except FVIII activity assays)
Mikhail Ovanesov, PhD (<i>MO</i>); OTAT/DPPT/HB	Potency control (3.2.S.4-5, 3.2.P.4-6) & FVIII activity testing (5.3.1.4)
Yideng Liang, PhD (<i>YL</i>); OTAT/DPPT/HB	Stability of DS and DP (3.2.S.7 & 3.2.P.8)
Ze Peng, PhD (<i>ZP</i>); OTAT/DPPT/HB	Adventitious Agents safety evaluation and validation of viral clearance (3.2.S.2.3, 3.2.P.4.5 & 3.2.A.2)
Mark Verdecia, PhD (<i>MV</i>); OTAT/DPPT/HB	Reference Standards or Materials (3.2.S.5 and 3.2.P.6)
Haarin Chun, PhD (<i>HC</i>); OTAT/DPPT/HB	DS and DP manufacture (3.2.S.2 & 3.2.P.3)

7. INTER-CENTER CONSULTS

Reviewer/Affiliation	Topic (Module 5 Appendix Section)	In agreement with consult conclusion /recommendations
Idalia E. Rychlik, Center for Drug Evaluation and Research (CDER)/ Office of Surveillance and Epidemiology (OSE) & Office of Medication Error Prevention and Risk Management (OMEPRM) / Division of Medication Error Prevention and Analysis (DMEPA)	Product Information/ Prescribing Information (A), Previous DMEPA Reviews & Information Requests (B), Summative Usability Test Report, Differentiation Tasks (C) & Human Factors Validation Test Conclusive Report (D)	Yes

8. SUBMISSIONS REVIEWED

Date Received	Submission/Amendment	Comments (reviewer)
04/27/2018	STN 125671/06 (response to IR)	Acceptable (AS)
05/18/2018	STN 125671/07 (response to IR)	Acceptable (AS)
08/23/2018	STN 125671/24 (response to IR)	Acceptable (AS)
08/27/2018	STN 125671/25 (response to IR)	Acceptable (ZP)
09/27/2018	STN 125671/30 (response to IR)	Acceptable (YL)
10/05/2018	STN 125671/32 (response to IR)	Acceptable (AS)
10/10/2018	STN 125671/33 (response to IR)	Acceptable (AS)
10/18/2018	STN 125671/35 (response to IR)	Acceptable (AK, YL)
11/02/2018	STN 125671/37 (response to IR)	Acceptable (AK, YL)
11/16/2018	STN 125671/41 (response to IR)	Acceptable (YL)
11/30/2018	STN 125671/45 (response to IR)	Acceptable (AS)
12/10/2018	STN 125671/46 (response to IR)	Acceptable (AS)
12/11/2018	STN 125671/48 (response to IR)	Acceptable (AK, YL)
12/20/2018	STN 125671/52 (response to IR)	Acceptable (MV)
12/20/2018	STN 125671/53 (response to IR)	Acceptable (AK)
12/21/2018	STN 125671/55 (response to IR)	Acceptable (AK, YL)
01/16/2019	STN 125671/57 (response to IR)	Acceptable (AS, YL, HC)

9. REFERENCED REGULATORY SUBMISSIONS

Submission Type & #	Holder	Referenced Item	Letter of Cross-Reference	Comments/Status
IND 14410	Novo Nordisk Inc.	GlycoPEGylated Coagulation Factor VIII (Recombinant) [N8-GP]	N/A	Ongoing
Drug Master Files (DMF) (b) (4)	(b) (4)	Lyophilization Stopper 13 mm grey	Yes	No DMF review required, information pertinent to container closure is provided in the BLA
DMF (b) (4)	(b) (4)	Syringe barrel 5 mL	Yes	No DMF review required, information pertinent to container closure is provided in the BLA

Submission Type & #	Holder	Referenced Item	Letter of Cross-Reference	Comments/Status
DMF (b) (4)	(b) (4)	Rubber plunger 5 mL	Yes	No DMF review required, information pertinent to container closure is provided in the BLA
DMF (b) (4)	(b) (4)	Syringe closure system 5 mL	Yes	No DMF review required, information pertinent to container closure is provided in the BLA
510(k) (b) (4)	(b) (4)	Vial adapter - (b) (4)	Yes	No DMF review required, information pertinent to vial adapter is provided in the BLA

10. REVIEWER SUMMARY AND RECOMMENDATION

A. Executive summary

This review is an assessment of CMC information from the product quality perspective in Biologics License Application (BLA) under STN 125671 submitted by Novo Nordisk, Inc. (Novo). The product is Antihemophilic Factor (Recombinant), GlycoPEGylated /exei (INN: turoctog alfa pegol), and its proprietary name for the US market is ESPEROCT. The active ingredient of ESPEROCT is a pegylated (PEG) recombinant B-domain-deleted (BDD) analogue of human coagulation factor VIII (rFVIII). In this molecule, the B-domain is replaced with a polypeptide containing a site for O-glycosylation. During production, a 40-kDa polyethylene glycol moiety is attached enzymatically to this glycan resulting in production of rFVIII-PEG.

ESPEROCT is indicated for use in adults and children with hemophilia A for (i) on-demand treatment and control of bleeding episodes, (ii) perioperative management of bleeding and (iii) routine prophylaxis to reduce the frequency of bleeding episodes. The drug product (DP) represents a lyophilized powder supplied in single-dose glass vials containing 500, 1000, 1500, 2000, and 3000 International Units (IU) of FVIII activity. The product is intended for intravenous administration after reconstitution with 0.9% sodium chloride solution supplied in pre-filled syringes. ESPEROCT was developed under Investigational New Drug application (IND) 14410, *GlycoPEGylated Coagulation Factor VIII (Recombinant) [N8-GP]*.

Herein, we present a consolidated review of the information provided in the original BLA and subsequent amendments, which were submitted upon the Agency's requests for additional information (IRs). As a result of the review, the manufacturing process for ESPEROCT is found adequately validated and controlled at the commercial scale to ensure consistent manufacture of the commercial product that meets release specifications. The manufacturing process provides sufficient margin of safety regarding adventitious agents. All CMC reviewers conclude that the Applicant has provided sufficient CMC data and information to support the identity, quality, purity, safety, and potency of ESPEROCT.

B. Recommendation

The CMC (Product) reviewers recommend **APPROVAL** of the BLA under STN 125671/0. The manufacturing process for ESPEROCT is considered adequately validated and controlled. No post-marketing requirement (PMR) or post-marketing commitment (PMC) studies are recommended. There are no lot release requirements for this product as it is well-characterized product manufactured using recombinant DNA technology (60 FR 63048-63049 publication, December 8, 1995). The major production facilities to be approved are the following.

- Novo Nordisk (b) (4) (production of Drug Substance (DS)).
- Novo Nordisk A/S, (b) (4) (production of lyophilized Drug Product (DP)).
- (b) (4) (production of Solvent in pre-filled syringe).

Detailed review of facilities was performed by Office of Compliance and Biologics Quality, Division of Manufacturing and Product Quality (OCBQ/DMPQ).

11. SIGNATURE BLOCK

Reviewer/Title/Affiliation	Concurrence	Signature and Date
Andrey Sarafanov, PhD /Chemist /OTAT/DPPT/HB	Concur	
Alexey Khrenov, PhD /Senior Staff Fellow /OTAT/DPPT/HB	Concur	
Mikhail Ovanesov, PhD /Research Biologist /OTAT/DPPT/HB	Concur	
Yideng Liang, PhD /Biologist /OTAT/DPPT/HB	Concur	
Ze Peng, PhD /Biologist /OTAT/DPPT/HB	Concur	
Mark Verdecia, PhD /Staff Fellow /OTAT/DPPT/HB	Concur	
Haarin Chun, PhD /Staff Fellow /OTAT/DPPT/HB	Concur	
Natalya Ananyeva, PhD /Chemist (Team Leader) /OTAT/DPPT/HB	Concur	
Tim Lee, PhD / Supervisory Research Chemist (Branch Chief) /OTAT/DPPT/HB	Concur	
Basil Golding, MD / Supervisory Medical Officer (Division Director) /OTAT/DPPT	Concur	

REVIEW OF CTD

Module 3

3.2.S DRUG SUBSTANCE

3.2.S.1 General Information

3.2.S.1.1 Nomenclature

- Non-Proprietary Name (US Established Proper Name): Antihemophilic Factor (Recombinant), ClycoPEGylated / exei
- International Non-Proprietary name (INN): Turoctocog alfa pegol
- Proprietary Name (U.S. established name): ESPEROCT
- Company/laboratory code: Turoctocog alfa pegol
- Chemical Abstract Service (CAS) registry number: (b) (4)
- CAS index name: Blood-coagulation factor VIII (synthetic human N8 heavy chain), compd. with blood-coagulation factor VIII (synthetic human N8 light chain), 40-kilodalton pegylated
- *Other names:* (b) (4) N8-GP; NNC 0129-0000-1003

3.2.S.1.2 Structure

The recombinant FVIII protein (rFVIII) in ESPEROCT is expressed in a Chinese Hamster Ovary (CHO) cell line using recombinant DNA technology. The human FVIII gene is genetically modified to express rFVIII in which the B-domain is replaced with a (b) (4)

(b) (4) cleavages and for O-glycosylation (b) (4). The 40-kilodalton (kDa) PEG moiety is enzymatically conjugated to the O-glycan. The resulting molecular mass of rFVIII-PEG is (b) (4). Similar to native FVIII, the molecule is composed of the (b) (4) heavy chain (HCh) and the (b) (4) light chain (LCh), which are bound non-covalently. Both (b) (4) have post-translation modifications (PTMs), which include (b) (4) that follows the PTMs pattern of native FVIII.

3.2.S.1.3 General Properties

In the blood circulation, the PEG moiety of rFVIII (b) (4) extends the molecule's half-life to (b) (4) hours compared to (b) (4) hours for native FVIII (b) (4) times). In the circulation, rFVIII-PEG is activated by thrombin (b) (4) with the attached PEG moiety dissociates from the activated rFVIII (rFVIIIa). This molecule is similar in structure and function to native FVIIIa playing a co-factor role in the intrinsic coagulation pathway to achieve hemostasis.

3.2.S.2 Manufacture

3.2.S.2.1 Manufacturer(s)

The Drug Substance (DS) is manufactured at Novo's facility in (b) (4) and tested according to specifications at Novo's facility in (b) (4). The facility-related information was reviewed by Office of Compliance and Biologics Quality, Division of Manufacturing and Product Quality (OCBQ/DMPQ).

3.2.S.2.2 Description of Manufacturing Process

The manufacturing process for DS includes (b) (4). These include the following steps.

(b) (4)

(b) (4)

(b) (4)

(b) (4)

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(b) (4)

[Redacted]

[Redacted]

[Redacted]

(b) (4)

[Redacted]

[Redacted]

Storage and Shipping

This information was reviewed by OCBQ/DMPQ.

Overall Reviewer's Assessment of Section 3.2.S.2:

The manufacturing process for DS and batch and scale definitions are described in sufficient detail and are acceptable as submitted. All production steps until Step (b) (4) were previously approved for the manufacturing of NOVOEIGHT (STN 125466). Information on DS manufacturers, filling procedures, equipment qualification and cleaning, storage and transportation of intermediates and DS were reviewed by OCBQ/DMPQ.

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(b) (4)

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(b) (4)

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(b) (4)

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Overall Reviewer's Assessment of Section 3.2.S.2.3:

Control of all materials used in production of ESPEROCT is adequate. No deficiencies were identified. The characterization of (b) (4) is consistent with ICH Guidelines Q5A(R1), Q5B and Q5D. The biological materials were found safe with regard to adventitious agents

(section 3.2.A.2). Though retrovirus-like particles (RVLs) were detected (upon (b) (4) [redacted]), their presence is a known phenomenon for CHO-derived cells and these particles are considered non-pathogenic. Furthermore, there are two dedicated virus removal steps in the manufacturing process that ensures safety of ESPEROCT. Thus, the information provided is acceptable as submitted. All materials used until step (b) (4) [redacted] were previously approved for manufacturing NOVOEIGHT.

3.2.S.2.4 Controls of Critical Steps and Intermediates

The control strategy was developed in accordance with ICH Guideline Q11, *Development and Manufacture of Drug Substances. Chemical Entities and Biotechnological/Biological Entities*. This strategy was based on the determination of critical quality attributes (CQA) and process parameters, which may affect CQAs. The CQA were rated according to a severity score (from 1 to 5). The most important CQAs (severity scores of 4-5) were (b) (4) [redacted]. Risk assessment identified that all process steps have a potential impact on CQA, with high severity score (≥ 3 , section 3.2.S.4.5). Respective in-process controls with acceptable ranges/limits and relevant analytical procedures were developed to ensure preservation of CQAs. The most critical steps are: (b) (4) [redacted].

(b) (4) [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

(b) (4) [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

(b) (4)

Overall Reviewer's Assessment of Section 3.2.S.2.4:

The information for controls of the manufacturing process is acceptable as submitted.

3.2.S.2.5 Process Validation and/or Evaluation

The validation strategy included (i) Process Design, (ii) Process Performance Qualification and (iii) Continued Process Verification.

Process Performance Qualification (PPQ) was performed at the commercial scale and intended manufacturing site under prospective process validation protocols and was based on monitoring for compliance of process parameters and in-process tests with respective acceptance ranges or limits (section 3.2.S.2.4).

(b) (4)

During the PPQ process, the respective operation parameters were monitored, respective intermediates were sampled and tested according to respective standard operation procedures (SOPs), and the resulting DS batches were tested versus release specifications. All acceptance criteria were met. The process deviations were investigated for root causes and considered to not affect the product quality and process validation. No reprocessing of an intermediate is used at any of the process steps. Validation of DS formulation, filling, packaging, shipping, and cleaning/sterilization of equipment was reviewed by OCBQ/DMPQ. The manufacturing process was qualified as consistent and reproducible. A plan for Continued Process Verification during future production of DS was established (section 3.2.P.3.5).

Overall Reviewer's Assessment of Section 3.2.S.2.5:

Novo's validation strategy for the DS manufacturing process is consistent with the recommendations of ICH Guidelines Q7, Q8 and Q11. The experimental data generated during process validation studies are all satisfactory and supportive of process consistency. The information on process validation is acceptable as submitted.

3.2.S.2.6 Manufacturing Process Development

During the process design and development, the critical quality attributes (CQA) of DS and process parameters which affect COA were determined, and DS specifications were established (sections 3.2.S.2.4 and 3.2.S.4.5). The following changes were introduced.

(b) (4)

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(b) (4)

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The process development regarding formulation, filling, packaging, shipping, cleaning, sterilization, *etc.* was reviewed by OCBQ/DMPQ.

Overall Reviewer's Assessment of Section 3.2.S.2.6:
The information for manufacturing process development is acceptable as submitted. Novo provided sufficient data to demonstrate comparability of DS material from the development and clinical stages to the commercial manufacturing processes.

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

(b) (4)

(b) (4)

3.2.S.4 Control of Drug Substance

3.2.S.4.1 Specification(s) and 3.2.S.4.5 Justification of Specification(s)

The specifications for DS were established in accordance with ICH Guideline Q6B. The specification parameters were selected from the CQAs which were identified based on risk analyses and their severity score (refer to 3.2.S.2.4). Acceptance ranges/limits were established based on manufacturing capability, clinical outcome, analytical variability, and stability data. The specifications and justification for each parameter are provided in the following table.

Table 3.2.S.4.1-1. Specifications for Drug Substance and Their Justification

(b) (4)

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

(b) (4)

3.2.S.4.2 Analytical Procedures and 3.2.S.4.3 Validation of Analytical Procedures

This information for all analytical methods except for Potency was reviewed by OCBQ/DBSQC. The review of FVIII activity (Potency) determination is provided below.

FVIII activity can be assessed either by a chromogenic substrate FVIII activity assay (CS) which is based on (b) (4) chromogenic substrate or by a one-stage clotting FVIII activity assay (OC) which is based on (b) (4) assay. The use of CS was supported by clinical trials. The FDA clinical team found the results of the clinical studies to be supportive of the proposing dosing. Because the clinical trial product was labeled with the CS assay, and the proposed validated potency assay is traceable to the Potency assays used in clinical trials, the proposed CS assay is acceptable.

Thus, FVIII activity in (b) (4) is determined by a chromogenic assay described in the (b) (4) FVIII activity is expressed in IU, which are traceable to the (b) (4) WHO FVIII IS (b) (4). The method (b) (4) was validated using an (b) (4) Specificity, Linearity,

Accuracy, Precision (Repeatability and Intermediate Precision), Range and Robustness. All acceptance criteria were met, and the assay was concluded to be suitable for determination of FVIII activity (Potency) in (b) (4) DP.

Overall Reviewer's Assessment of Sections 3.2.S.4.2 and 3.2.S.4.3:

Selection of a chromogenic assay for determination of FVIII activity (Potency) in (b) (4) is sufficiently justified and suitable for the intended purpose. Validation of the method was performed in accordance with the ICH Q2 (R1) guideline and is acceptable as submitted. Application of CS assay for DP potency labeling and potential discrepancies between CS and OC potency values in testing post-infusion plasma samples is discussed under 3.2.P.5.2-3.2.P.5.3 and 5.3.1.4. Review of other methods used for release specifications of (b) (4) was performed by OCBQ/DBSQC and all methods were found adequately validated and suitable for their intended use.

3.2.S.4.4 Batch Analyses

Novo manufactured (b) (4) batches of the DS. A laboratory scale batch was used for preclinical studies. Out of (b) (4) clinical process batches, (b) (4) batches were used for phase 1 and phase 3 studies, and (b) (4) batches were engineering ones. Out of (b) (4) commercial process batches, (b) (4) batches were used for clinical phase 3 studies, and (b) (4) batches were engineering ones. PPQ campaign included (b) (4) batches manufactured by commercial process; the batch records overview is provided under section 3.2.R below. Batch analysis data were provided for all produced DS batches in the BLA. These batches were produced between (b) (4). The manufacture dates were evenly distributed without major gaps. Technical batches were manufactured using commercial process from (b) (4) and clinical batches were manufactured from (b) (4). During 2015-2016, the clinical batches were manufactured by both clinical and commercial processes at different facilities.

All batches met specifications acceptance criteria at time of analysis. As evident from the provided data, the acceptance criteria were evolving to become more stringent, and additional specifications parameters were added along the production of further DS batches as discussed in review of 3.2.S.4.1 *Specification(s)* and 3.2.S.4.5 *Justification of Specification(s)*. While no trends of data in graphical format were provided, the graphs submitted in section 3.2.S.4.5 demonstrated improvement in manufacturing consistency over time.

Overall Reviewer's Assessment of Sections 3.2.S.4.2, 3.2.S.4.3 and 3.2.S.4.4:

The batch analysis data demonstrate improvements in the manufacturing process during its development and adequate control strategy. The information is acceptable as submitted.

3.2.S.5 Reference Standards or Materials

Novo uses two-tier reference material scheme for ESPEROCT, with Primary Reference Material (PRM) and Secondary Reference Material (SRM). The tests using reference material include

Identity, Potency and Protein Content. PRM is used as a calibrator for SRM, and the SRM is used in routine QC testing. The PRM batch (b) (4) and SRM batch (b) (4) were manufactured by the commercial process as a (b) (4) IU/vial ESPEROCT DP lot (b) (4). This DP lot was released against the phase 3 ESPEROCT DP specifications valid at the time of production. The lot (b) (4) also fulfils the current specifications. Thus, this lot of DP was considered suitable for use as SRM and PRM. Based on it, a part of the lot (b) (4) was allocated to be used as PRM, and the other part of the lot was allocated to be used as SRM. Other reference materials used during DP development are described in the table below.

Table 3.2.S.5-1. ESPEROCT Primary and Secondary Reference Materials

Established for use in clinical:		Phase 1	Phase 3	Phase 3 and commercial use
Primary reference material (PRM)	Batch	(b)	(4)	
	Used as			
	Formulation			
	Valid in the period			
Secondary reference material (SRM)	Batch	(b)	(4)	
	Used as			
	Formulation			
	Valid in the period			
Secondary reference material (SRM)	Batch	(b)	(4)	
	Used as			
	Formulation			
	Valid in the period			

The Applicant provided qualification reports for both PRM and SRM for use in respective assays. The studies included establishing (b) (4) . Additional characterization of (b) (4) was performed along with testing for (b) (4) .

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

The submission includes protocols for establishing future batches for PRM and SRM. These protocols follow the same approach as that used for qualification of the current reference materials. The new batches of PRM will be produced and qualified in due time before the expiry date is reached or before the PRM batch is depleted. New reference materials will be established

according to the protocol for establishment of the PRM and released according to the specification in the protocol.

Overall Reviewer’s Assessment of Section 3.2.S.5:

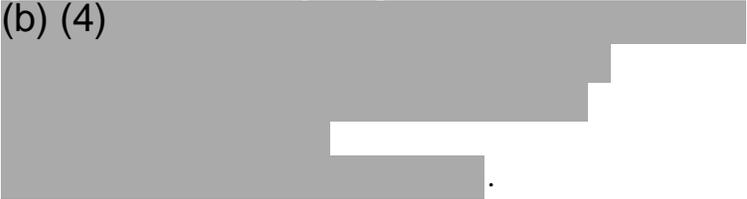
Upon initial review, several deficiencies were identified. In particular, trends for (b) (4) in (b) (4) were observed for the early batches of PRM and SRM (b) (4) over time (during the storage at (b) (4)). The reviewer suggested to investigate the root cause and provide additional data supporting acceptance of the current standards. In Novo’s response (STN 125671/52) they provided the following.

- (b) (4)
- 

In conclusion, the current reference materials are appropriately qualified, and storage conditions to maintain stability were established. The proposed protocols for qualifying future reference materials are appropriate. Altogether, the information is acceptable as submitted.

3.2.S.6 Container Closure System

The container closure system used for DS is a (b) (4) container manufactured from (b) (4) and provided sterile from the vendor. The container/closure system complies with the following compendia:

- (b) (4)
- 

The compatibility of the container with DS was reviewed by OCBQ/DMPQ except for the evaluation of leachables. In the submitted study results, leachables were initially assessed via extractables, and then quantitated and assessed for risk.

(b) (4)



(b) (4)



Overall Reviewer's Assessment of Section 3.2.S.6:

The information for Container Closure System is acceptable as submitted. Additional review comments are the following:

1) FDA does not require evaluation of leachables at particular steps of manufacture but requires evaluation of those in final DP container under real-time storage throughout the DP shelf-life. However, the performed study is useful to support the suitability of the container for storage of DS.

2) (b) (4)



3) No elemental extractables were evaluated for the DS container. It is acceptable considering that there are no regulatory requirements to perform extractables studies on intermediate process steps. Assessment of elemental leachables in the final DP is discussed in section 3.2.P.2.4.

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

3.2.P DRUG PRODUCT (Lyophilized Powder)

3.2.P.1 Description and Composition of the Drug Product

DP is provided as a sterile lyophilized powder in five nominal dosage strengths of 500, 1000, 1500, 2000 and 3000 international units (IU) of FVIII activity (potency) per vial. ESPEROCT DP is intended for intravenous administration after reconstitution with 0.9% sodium chloride solution provided in a prefilled syringe with (b) (4) of the diluent.

The vial for the lyophilized powder is a 5 mL vial for all product presentations made of (b) (4) glass with chlorobutyl rubber stopper and sealed with a snap-off cap made of aluminum and plastic. The vial adapter is a sterile, disposable device to allow for the transfer of reconstituted product into syringe (see section 3.2.P Solvent).

3.2.P.2 Pharmaceutical Development

3.2.P.2.1 Components of the Drug Product

Drug Substance

The active ingredient of (b) (4), which physicochemical and biological properties are reviewed in section 3.2.S.3.

Excipients

All excipients are of non-animal origin and pharmacopeial quality (b) (4), and used in other medical products for parenteral use, in particular, NOVOEIGHT (STN 125466). Upon reconstitution, the excipients composition is the same for all DP dosages. The final concentrations and functions of excipients are the following: L-Histidine (a (b) (4)), Sucrose (b) (4), Polysorbate 80 (b) (4), Sodium Chloride (b) (4), L-Methionine ((b) (4)) and Calcium Chloride (b) (4). Also, (b) (4) are used for (b) (4) adjustment of the formulation buffer.

3.2.P.2.2 Drug Product

Formulation Development

The formulation development was aimed to stabilize lyophilized FVIII and was based on Novo's experience with NOVOEIGHT and other licensed coagulation factors products. Justification of choice of each excipient is provided in the BLA. In particular, L-Methionine was used as an (b) (4), and Sucrose, Calcium and Polysorbate 80 were also used for FVIII (b) (4). During product development, the same formulation was used for non-clinical studies, phase 1 and phase 3 clinical trials (the section contains a list of (b) (4) clinical lots of DP). To secure physicochemical stability of the active ingredient, rFVIII-PEG, Potency, Purity, (b) (4), Protein content and (b) (4) were monitored during formulation development and shown to be consistent.

Overages

An overage of (b) (4) of rFVIII-PEG in all product dosages was determined to be sufficient to compensate for loss during lyophilization process and ensure the labeled potency. This overage has no impact on the product safety and efficacy.

Physicochemical and Biological Properties

The active ingredient in (b) (4) DP is rFVIII-PEG. The basic physicochemical and biological properties of rFVIII-PEG are discussed in section 3.2.S.3. At the same time, the following trends were observed during DP storage: increase of FVIII (b) (4), and respective decrease in Purity and FVIII activity. During stability study (section 3.2.P.8), respective acceptance limits for those parameters were determined.

3.2.P.2.3 Manufacturing Process Development

The manufacturing process was optimized from the initial lab scale to the final commercial scale production. This also included development of formulation and storage conditions, changes in facilities location (transfer of process to (b) (4) facility). Comparability of DS manufactured by clinical and commercial processes is discussed under section 3.2.S.2.6. The development of filling, lyophilization, packaging, shipping, cleaning and sterilization processes was reviewed by OCBQ/DMPQ.

3.2.P.2.4 Container Closure System

The Container/Closure System (CCS) for lyophilized DP represents 5-mL (b) (4) glass vial (b) (4) closed with 13-mm chlorobutyl rubber stopper coated with a polymer (b) (4) aluminum cap, and plastic snap-off. Each vial contains a single 4-mL dose after reconstitution. This information is also described in section 3.2.P.7 (see below).

Compatibility of the CCS with DP was studied regarding sorption, discoloration and stability of DP. This included testing of loosened stopper and powdered lyophilized cake, stored in (b) (4) position at (b) (4) 6 months that simulated the worst-case storage condition. Upon testing, the major specification parameters were found to be stable, except for (b) (4), (b) (4). These parameters were increased, while no sorption or discoloration was detected. The following compatibility studies were performed regarding extractables and leachables (E&L).

Extractables

The study was performed for rubber stopper only. In a study for (b) (4)

including NOVOSEVEN, NOVOEIGHT and REFIXIA. In a study for elemental extractables, the (b) (4)

No elemental extractables were found.

Leachables

In accelerated study, the vials (b) (4)

. Elemental extractables were not analyzed (see reviewer's comment below).

In a long-term stability study for organic leachables, the vials (b) (4)

Thus, the container closure system was considered to be safe for intended use.

3.2.P.2.5 Microbiological Attributes

This section was reviewed by OCBQ/DMPQ.

3.2.P.2.6 Compatibility

Compatibility of DP with the container closure system was also tested as stability of solution after reconstitution of the lyophilized DP (500 IU and 3000 IU dosage strengths). The solutions were tested for the major quality-indicating parameters: Appearance, (b) (4), Protein Content, (b) (4), Purity, Potency, Particulate Matter and (b) (4) for up to (i) 24 h when stored at (b) (4) and (ii) (b) (4) when stored at (b) (4). The results showed that all tested parameters, except for (b) (4), remained constant during 24-h storage at (b) (4). During the proposed in-use period of 4 h, only (b) (4) demonstrated a slight (b) (4) (up to (b) (4), section 3.2.P.8.3). It was concluded that DP is compatible with the solvent, container closure system, vial adapter and pre-filled syringe.

Overall Reviewer's Assessment of Section 3.2.P.2:

Information on pharmaceutical development was reviewed by OTAT and DMPQ reviewers. In the original application, assessment of extractables was limited to the stopper and the results were satisfactory, but no data for extractables from glass container were provided as justified by their expected absence in glass. The absence of this assessment was still acceptable, as not required by FDA Guidance for Industry, Container Closure Systems for Packaging Human Drugs and Biologics, 1999 (P. 23). This guideline requires that only for stopper as "for elastomeric packaging component (container closure) for injectable drug products, extractables should be identified whenever possible...".

However, glass is known to be a source of elemental (metal) compounds but no assessment of elemental leachables in DP was provided in the initial submission. This was justified by their absence “in other approved lyophilized hemophilia drug products using the same combination of vial and rubber stopper”. However, the reviewer had concern that different manufacturing process may result in appearance of different leachables in DP. To address this concern, Novo performed additional studies to analyze elemental leachables in the container system and showed their acceptable levels in the product (STN 125671/7). Thus, the assessment of E&L in the Container Closure System was found acceptable.

Altogether, the information on pharmaceutical development with regard to formulation development and compatibility of DP with the diluent, container closure system, vial adapter and syringe for administration is acceptable.

3.2.P.3 Manufacture

3.2.P.3.1 Manufacturer(s)

The manufacturing of the Drug Product (DP) is performed at (b) (4) . (b) (4) other facilities, located in (b) (4) , are responsible for storage and quality control of all materials, in-process and release analytical testing, labelling, secondary packaging, and storage of finished ESPEROCT DP. This information was reviewed by OCBQ/DMPQ.

3.2.P.3.2 Batch Formula

In final DP vials, the amount active ingredient (rFVIII-PEG) varies respectively product dosage (500-3000 IU/vial), whereas content of all excipients is the same (section 3.2.P.1). The section provides amounts of all excipients per (b) (4) of liquid product, and per its minimal and maximal batch sizes (b) (4)

Overall Reviewer’s Assessment of Sections 3.2.P.3.1 and 3.2.P.3.2:

The information on DP manufacturers was reviewed by OCBQ/DMPQ. The information on batch formula is acceptable as submitted.

3.2.P.3.3 Description of Manufacturing Process

The manufacturing process for DP includes the following steps.

(b) (4)



(b) (4)

2. Sterile filtration

(b) (4)

3. Filling

(b) (4)

4. Lyophilization

(b) (4)

5. Capping

(b) (4)

6. Visual inspection

The DP is visually inspected, and then sampling of the vials is performed for quality control testing. The time limit for inspection is (b) (4)

7. Storage

Finished DP is transferred to the warehouse and stored protected from light at 2–8°C.

(b) (4)

Packaging

Respective SPOs are followed for labelling, packaging and shipping. The packaging process is performed manually and/or automatically. A vial with DP and pre-filled syringe with solvent are labelled and packed together with the vial adapter in cartons provided with imprint of batch

number and expiry date. The information about filling, lyophilization, shipping, equipment qualification and cleaning; endotoxin and bioburden control was reviewed by OCBQ/DMPQ.

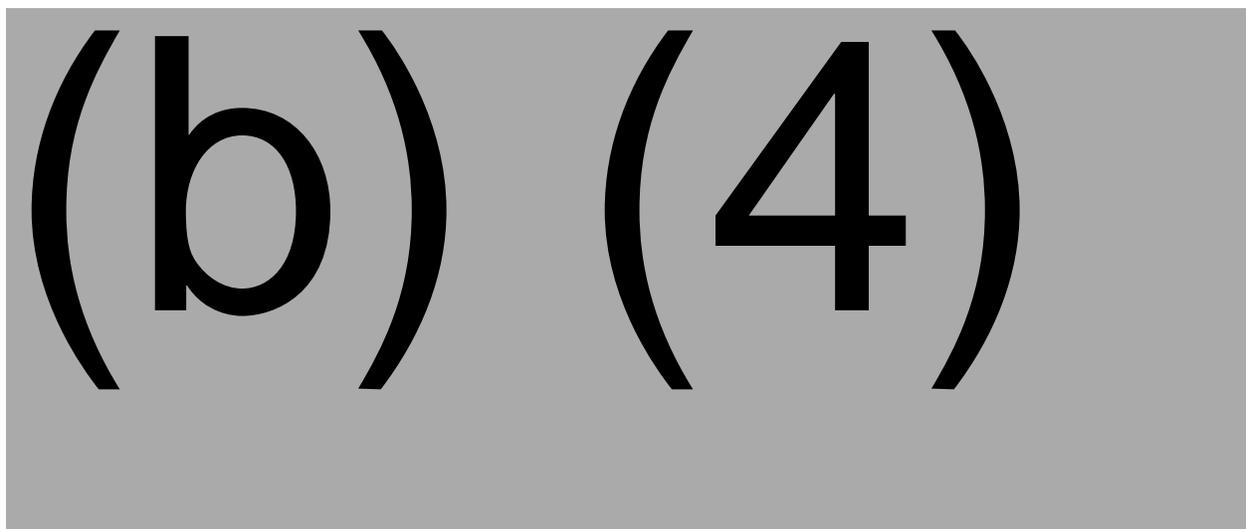
Overall Reviewer’s Assessment of Section 3.2.P.3.3:

The description of the manufacturing process and respective control parameters is sufficiently detailed. The corresponding process parameters, controls and hold times for intermediates appear adequate to ensure product quality (section 3.2.P.3.4). Thus, the information on the DP manufacturing process is acceptable as submitted.

3.2.P.3.4 Controls of Critical Steps and Intermediates

Based on risk assessment, the critical process steps in the DP manufacture are (b) (4). The respective controls are the following.

Table 3.2.P.3.4-1. Critical Steps and In-Process Control of Drug Product manufacture



This section also contains information for justification of attachment of a label with scale to the pre-filled syringe with Solvent. The information for critical process steps was also reviewed by OCBQ/DMPQ.

Overall Reviewer’s Assessment of Section 3.2.P.3.4:

The information on critical steps is acceptable as submitted. The respective in-process controls seem to be adequate to ensure product quality and manufacturing process consistency. In-process controls for the Lyophilization step were reviewed by OCBQ/DMPQ and found adequate.

3.2.P.3.5 Process Validation and/or Evaluation

The process validation strategy comprised (i) Process Design, (ii) Process Performance Qualification and (iii) Continued Process Verification.

1. Process Design

Process design included manufacturing DP lots for clinical phase 3 trials and process evaluation in full manufacturing scale in the (b) (4) facility. Prior knowledge from the manufacture of other licensed lyophilized parenteral products (in particular, NOVOEIGHT) was used as the basis in the design of the manufacturing process for rFVIII-PEG. The process design included evaluation of (i) (b) (4)

2. Process Performance Qualification

The process validation used a (b) (4) approach and involved production of (b) (4) consecutive lots (batches) of DP (500 IU, 1000 IU, 2000 IU and 3000 IU); (b) (4) with the (b) (4) that covered the manufacturing range of (b) (4) batch size as follows.

Table 3.2.P.3.5-1. **Manufactured PPQ lots of Drug Product**

Drug Product	Batch size	Drug substance batch no.	Drug product lot no.	Date of manufacture	Use
500 IU	(b) (4)				PPQ, Clinical trials Stability Studies
2000 IU	(b) (4)				PPQ, Clinical trials Stability Studies
3000 IU	(b) (4)				PPQ, Clinical trials Stability Studies
1000 IU	(b) (4)				PPQ, Stability Studies

The executed batch records for these PPQ lots are provided in section 3.2.R. For all DP lots, all in-process controls and results of release testing versus DP specifications met the acceptance criteria. The manufacturing process was considered validated. The information about validation of formulation, filling, lyophilization, packaging, shipping, cleaning and sterilization was reviewed by OCBQ/DMPQ.

3. Continued Process Verification

Continued process verification plan was developed as described in section 3.2.P.5.6 *Control Strategy for Drug Product*. According to the plan, extended monitoring of the manufacturing process performance will be continued with (b) (4) review of manufacturing data and risk assessment of test parameters. This plan also includes production of a post-PPQ (b) (4) 1500 IU and its monitoring for stability.

Overall Reviewer’s Assessment of Section 3.2.P.3.5:

The process validation program is consistent with the recommendations of ICH Guidelines Q7, Q8 and Q11, and all results of in-process and release testing are satisfactory. However, the reviewers had concern that the (b) (4) approach (i.e., dosage strength of 1500 IU was not produced in the PPQ study) was not adequately supported by data: the data at the lower end were found insufficient considering that (b) (4) 1000 IU was manufactured at commercial scale and only 12-month stability data are currently available for PPQ lots at long-term storage conditions. In the course of review, a request was conveyed to the Applicant to provide more information in support of the (b) (4) approach. Novo provided stability trends graphs from a completed “accelerated” stability study (6-month (b) (4) for primary, supportive and PPQ lots, and a post-PPQ (b) (4) 1500 IU dosage strength (total of (b) (4) DP lots). All parameters were within specifications with comparable stability profiles observed for all dosage strengths (section 3.2.P.8). These results further supported the process validation for all dosage strengths, including 1500 IU. Altogether, the PPQ data demonstrated consistency and reproducibility of the manufacturing process and the information on validation of DP manufacturing process is acceptable.

3.2.P.4 Control of Excipients

The excipients used in ESPEROCT are L-Histidine, Sucrose, Polysorbate 80, Sodium Chloride, L-Methionine, and Calcium chloride (b) (4) . All of those are of non-animal origin, have compendial specifications (b) (4) and are used in other medical products for parenteral use, in particular, NOVOEIGHT (STN 125466). Therefore, all these excipients are considered to be safe.

Overall Reviewer’s Assessment of Section 3.2.P.4:

The information provided for excipients is acceptable as submitted.

3.2.P.5 Control of Drug Product

3.2.P.5.1 and 3.2.P.5.6 Specification(s) and Justification of Specification(s)

The DP specifications were justified based on: (i) manufacturing experience gained during the process development, (ii) determination of CQAs of ESPEROCT and (iii) process parameters which may impact those. The CQAs were rated according to the severity score (from 1 to 5). The high-score CQAs are (b) (4) , Potency, (b) (4) , and Bacterial Endotoxins. The specifications and detailed justification for each parameter is provided in the following table. Phase 3 clinical specifications in the table refer to the Phase 3 specifications Version 7.0, which was used for most of clinical batches. Versions 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 were also used and referenced in section 3.2.P.5.4 (*Batch Analyses*).

Table 3.2.P.5.1-1. Release Specifications for Drug Product and their Justification

Test Parameter (Attribute)	Analytical Procedure	Final Acceptance Criteria	Justification for Specification	Phase 3 Clinical Lot Acceptance Criteria (if different from commercial)	PPQ/Validation Lots Acceptance Criteria (if different from commercial)
Appearance of powder	Visual inspection	White to off-white lyophilizate	Based on manufacturing experience		
Reconstitution time/solubility	Visual inspection	(b) (4)	Based on results from (b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)	Based on results from (b) (4)	(b) (4)	(b) (4)
	(b) (4)	(b) (4)			
Appearance of solution	Visual inspection	Clear and colorless liquid, free from particles that are clearly detectable.	Based on manufacturing experience		
(b) (4)	(b) (4)	(b) (4)	Based on results from (b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)	Based on results from (b) (4)	(b) (4) %	(b) (4)
Protein content		(b) (4)	(b) (4)	(b) (4)	(b) (4)
		(b) (4)	(b) (4)	(b) (4)	(b) (4)
		(b) (4)	(b) (4)	(b) (4)	(b) (4)
		(b) (4)	(b) (4)	(b) (4)	(b) (4) :

Test Parameter (Attribute)	Analytical Procedure	Final Acceptance Criteria	Justification for Specification	Phase 3 Clinical Lot Acceptance Criteria (if different from commercial)	PPQ/Validation Lots Acceptance Criteria (if different from commercial)
		(b) (4)		(b) (4)	
Identity	(b) (4)		Based on characterization studies	Comparable to reference	
(b) (4)			Based on results from (b) (4)	For information only	(b) (4)
Purity			Based on results from (b) (4)	(b) (4)	(b) (4)
Potency (IU/vial)	Chromogenic assay		(b) (4)	(b) (4)	(b) (4)

Test Parameter (Attribute)	Analytical Procedure	Final Acceptance Criteria	Justification for Specification	Phase 3 Clinical Lot Acceptance Criteria (if different from commercial)	PPQ/Validation Lots Acceptance Criteria (if different from commercial)
Particulate matter	(b) (4)	(b) (4)	(b) (4)		
(b) (4)	(b) (4)	(b) (4)	Based on results from (b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)	(b) (4)		
Polysorbate 80	(b) (4)	(b) (4)	Based on results from (b) (4)		(b) (4)
Calcium	(b) (4)	(b) (4)	(b) (4)	For information only	
Sucrose	(b) (4)	(b) (4)	(b) (4)	For information only	
Bacterial endotoxins	(b) (4)	(b) (4)	Based on manufacturing experience and LOQ of the method	(b) (4)	(b) (4)
Sterility	(b) (4)	Sterile	(b) (4)		

Upon review of the original application, multiple deficiencies were found, and addressed as described below. Regarding Potency (a key parameter of Specifications), the upper limits of the release and shelf-life limits are identical, whereas the release lower limits are more stringent than the shelf-life lower limits.

Overall Reviewer’s Assessment of Sections 3.2.P.5.1 and 3.2.P.5.6:

I. Compared to DS specifications (section 3.2.S.4.1), the DP specifications acceptance limits are generally narrower. The additional parameters for DP are: Content of Excipients, Appearance, Reconstitution Time, Particulate Matter, and DP specifications do not have such parameters as Pegylation Profile, parameters to control glycosylation, Residual Pegylation Enzymes (b) (4), HCP, (b) (4)

II. Upon FDA request, Novo adjusted the release limits for Potency to ensure compliance with the shelf-life limits (b) (4). The shelf limits of (b) (4)

(b) (4) are typical for coagulation factor concentrate products on the U.S. market. These products are labeled with the actual (released) potency in addition to the nominal value declaration. Novo stated that this description is aligned with the (b) (4) although (b) (4). The release limits are set higher and take into consideration potency losses during lyophilization.

III. The specifications for DP are established in accordance with ICH Guideline Q6B. The parameters were selected from the CQAs and acceptance ranges/limits were established based on manufacturing capability, clinical outcome, analytical variability, and stability data. However, in the original BLA, Novo did not provide data or detailed justifications to support most of the specifications. Some acceptance criteria were established based on DS acceptance criteria with some data from DP manufacture, which was not appropriate. In particular, the following acceptance criteria were affected:

- a. *Reconstitution time/solubility*: no data and statistical justification were provided.
- b. (b) (4): no data and statistical justification were provided.
- c. (b) (4) no data and statistical justification were provided to support (b) (4).
- d. (b) (4): no test was provided to demonstrate the capability of the manufacturing process to meet the acceptance criteria. The level of (b) (4) in the clinical lots was significantly lower than the proposed acceptance limit.
- e. (b) (4): no test was provided to demonstrate the capability of the manufacturing process to meet the acceptance criteria. The level of (b) (4) in the clinical lots was significantly lower than the proposed acceptance limit.
- f. *Purity*: no test was provided to demonstrate the capability of the manufacturing process to meet the acceptance criteria. The purity in the clinical lots was significantly higher than the proposed acceptance limit.
- g. (b) (4): no data and statistical justification were provided.
- h. *Polysorbate 80*: no data and statistical justification were provided.
- i. *Bacterial Endotoxins*: results for testing endotoxins in DP were not provided.

Therefore, Novo was requested to provide the missing information and establish appropriately justified acceptance criteria. In the provided response (Amendment 125671/37), the information was still insufficient to justify the proposed acceptance criteria. However, the following issues were identified.

a. Reconstitution time/solubility

The proposed acceptance criterion (b) (4) was based on a (b) (4) which is not a legally recognized compendium in the US. Also, the applicability of this criterion to a chemically modified protein, such as rFVIII-PEG, was not established. Since anomalies in reconstitution time can be indicative of issues encountered during DP manufacture, this parameter needs to be well-controlled to ensure manufacturing consistency. Therefore, Novo was requested to provide the actual reconstitution times for all manufactured lots and establish a statistically justified acceptance criterion based on these data or justify the use of the current acceptance criterion.

b. (b) (4)

The proposed acceptance criterion of (b) (4) was not adequately justified. The data provided showed a (b) (4) at product release. To justify the (b) (4) (b) (4) Novo referenced a value of (b) (4) obtained in a study in which lyophilization was performed with (b) (4) (a procedure not currently used), and (b) (4) was observed. As such, the inclusion of these data in the justification was not appropriate. Also, the shelf-life specification was set at (b) (4) was the maximum value observed in stability studies under normal and accelerated conditions. There were no data that had established product safety, efficacy, quality and stability a (b) (4).

c. (b) (4)

The proposed acceptance criterion was not justified. The company stated that “During the drug product (b) (4) is not allowed. Therefore, the drug product specification limits must cover the (b) (4) range allowed for drug substance.” This statement was misleading and incorrect, as the formulation process includes (b) (4). In the *Pharmaceutical Development* section of the BLA regarding excipients, Novo stated “L-Histidine is included in a concentration of (b) (4) mg/mL which is shown sufficient to secure a stable (b) (4) during the proposed shelf life and in use”. As such, the acceptance criterion for (b) (4) in the DP must be established separately from that of the DS, based on statistical analysis of the release testing data.

d. (b) (4)

The reviewer disagreed with Novo’s approach to establish the acceptance criterion for (b) (4) in DP based on that for (b) (4). It was not clear how these (b) (4) were estimated; moreover, comparison of the data for the (b) (4) DP (b) (4) did not support the magnitude of the (b) (4)” and validity of the model. Therefore, the reviewer requested to justify and establish the acceptance criterion for (b) (4) in DP based on statistical analysis of release testing data.

f. *Purity*

The reviewer disagreed with the approach to establish the acceptance criterion for Purity of DP based on that for DS and (b) (4). It was not clear how these (b) (4) were estimated; moreover, comparison of the data for DS (b) (4) DP (b) (4) did not support the magnitude of these (b) (4) and validity of the model. Therefore, the reviewer requested to justify and establish the acceptance criterion for *Purity* in DP based on statistical analysis of release testing data.

g. (b) (4)

The proposed acceptance criterion was not justified. The (b) (4) (b) (4), which was used to establish the specification range, is not relevant to ESPEROCT. Also, it was not clear how “acceptable variation in excipient concentration” was estimated. Therefore, the reviewer requested to justify and establish the acceptance criterion for (b) (4) based on statistical analysis of release testing data.

These additional requests were sent to Novo, and the company provided the responses in Amendment STN 125671/48. The responses were acceptable except for the acceptance criterion for (b) (4) parameter. The reviewer still disagreed with the approach to establish the acceptance criterion for (b) (4) in DP based on that for DS and (b) (4). The data did not show consistent increase of (b) (4) level in DP over their level in DS. As such, unless proposed acceptance criterion for DP is based on the level of (b) (4) in the sourced DS lot, this approach was not appropriate. The reviewer again requested to justify and establish the acceptance criterion for (b) (4) in DP based on statistical analysis of release testing data. Based on this reviewer's analysis of the data provided in the Amendment, the limits of (b) (4) Release ((b) (4) Shelf-life) appeared to be justified acceptance criteria for this parameter. A request for that was sent to Novo on December 17, 2018. In Amendment STN 125671/51, the company accepted the reviewer's proposal regarding the acceptance criterion for (b) (4). Thus, all issues identified in the course of review were adequately resolved. The final DP specifications are considered acceptable to control the identity, quality, purity, potency, and safety of ESPEROCT.

3.2.P.5.2 and 3.2.P.5.3 Analytical Procedures and Validation of Analytical Procedures

In addition to description of the methods, the respective validation reports are provided in section 3.2.R. All methods, except for Potency, were reviewed by OCBQ/DBSQC. The review of Potency (FVIII activity) by Chromogenic Method is provided in sections 3.2.S.4.2-3.

Overall Reviewer's Assessment of Sections 3.2.P.5.2 and 3.2.P.5.3:

Selection of a chromogenic assay for Potency assignment in DP is suitable for the intended purpose. Validation of the method was performed in accordance with the ICH Q2 (R1) guideline and is acceptable as submitted. The review of Potency (FVIII activity) by Chromogenic Method is provided in sections 3.2.S.4.2-3. The use of CS and OC assay for testing post-infusion plasma samples and discrepancies between the two assays observed in clinical trials are discussed in section 5.3.1.4. Review of other methods used for release specification testing of DP was performed by CBER/OCBQ/DBSQC and all methods were found adequately validated and suitable for their intended use.

3.2.P.5.4 Batch Analyses

The company manufactured (b) (4) lots of ESPEROCT DP in (b) (4) dosage strengths (b) (4) of 500 IU, (b) (4) of 1000 IU, (b) (4) 2000 IU, and (b) (4) of 3000 IU. (b) (4) laboratory scale lots and (b) (4) scale (b) (4) 2000 IU were manufactured, and the rest of (b) (4) lots was produced by the commercial process. (b) (4) lots were used for pre-clinical studies and process engineering, with the rest used or planned to be used in clinical studies. The release analysis data for all lots are provided in the BLA. These lots were produced between (b) (4). The manufacture of 2000 IU batches was evenly distributed without major gaps. 500 IU lots were manufactured starting (b) (4), also evenly distributed. All 1000 IU and 3000 IU batches were manufactured in 2016-2017. The testing of all lots met specifications' acceptance

criteria relevant at the time of analysis. The executed batch records for (b) (4) PPQ lots of 500, 1000, 2000 and 3000 IU dosage strengths are overviewed in section 3.2.R below.

3.2.P.5.5 Characterization of Impurities

The review of process-related and product-related impurities is provided under 3.2.S.3.2 (Impurities in Drug Substance). The contribution of the manufacturing process downstream the DP production was further investigated in additional testing during process development and in subsequent stability studies.

Product-related impurities

The potential product-related substances and impurities are FVIII size and (b) (4), PEG forms, (b) (4)

In the performed studies, no new product-related impurities were found in DP that were generated during the manufacturing process or storage. A minor increase in (b) (4) was observed by (b) (4) in DP at release. These forms of rFVIII-PEG corresponded to higher forms of (b) (4). A minor increase in (b) (4) forms by (b) (4) was observed during DP storage. These forms correspond to rFVIII-PEG with (b) (4) at multiple sites in (b) (4). A minor increase of (b) (4) forms of rFVIII-PEG was detected by (b) (4). These forms correspond to rFVIII-PEG (b) (4).

Process-related impurities

(b) (4) used for several lyophilized drug products of Novo. No safety concerns related to the (b) (4) level in DP were identified. Review of this information is provided under review of section 3.2.P.2.4. *Container Closure System*. The review of microbiological impurities provided in section 3.2.P.2.5 was performed by OCBQ/DMPQ.

Overall Reviewer's Assessment of Sections 3.2.P.5.4 and 3.2.P.5.5:

As evident from the provided data in section Batch Analyses, the acceptance criteria evolved to be more stringent and additional specifications parameters were added as more lots were produced and more data became available (see review of 3.2.P.5.1 Specifications and 3.2.P.5.6 Justification of Specifications). In the original submission, no trending data in graphical format were provided under Batch Analysis. These trend analyses were requested, and the graphs submitted under section 3.2.P.5.6 and Amendments 125671/37, 125671/48, and 125671/51 demonstrated good manufacturing consistency.

No new product-related impurities were found due to the DP manufacturing process or storage. The levels of (b) (4), which showed slight increase due to the (b) (4) DP storage, are controlled in the DP specifications. The levels of the deamidated forms of rFVIII-PEG (which showed minor increase) and (b) (4) are considered to be safe. Altogether, control of impurities in ESPEROCT is adequate. The batch analysis data demonstrate consistency and reproducibility of the manufacturing process and development of adequate control strategy. The information provided is acceptable.

3.2.P.6 Reference Standards or Materials

This information is described under section 3.2.S.5 *Reference Standards or Materials*.

3.2.P.7 Container Closure System

The container closure system for DP includes glass (b) (4) vial, rubber stopper and snap-off cap. The stopper is produced from chlorobutyl rubber coated with polydimethylsiloxane. The container/closure system complies with the following compendia.

Vial

- (b) (4) : Glass Containers for Pharmaceutical Use (b) (4) glass).
- (b) (4) Containers – Glass (b) (4) glass).
- (b) (4) Test for Glass Containers for Injections.

Stopper:

- (b) (4) : Rubber Closures for Containers for Aqueous Parenteral Preparations, for Powders and for Freeze-dried Powders (b) (4) rubber).
- (b) (4) : Elastomeric Closures for Injection (b) (4) rubber).
- (b) (4) .

Compatibility of DP with the container closure system, including information about impurities (leachables) from the container, was reviewed under sections 3.2.P.2.4 and 3.2.P.2.6. The suitability of the container to DP, container closure integrity testing and other information was reviewed by OCBQ/DMPQ.

Overall Reviewer's Assessment of Section 3.2.P.7:

The information supports suitability of the container closure system for use with DP and is acceptable as submitted. The same container closure system is used by Novo for other lyophilized hemophilia drug products: NOVOSEVEN, NOVOEIGHT and REFIXIA.

3.2.P.8 Stability

3.2.P.8.1 Stability Summary and Conclusion and 3.2.P.8.3 Stability Data

The stability studies for lyophilized DP included: (i) supportive stability study, (ii) primary stability study, (iii) PPQ study, (iv) in-use (upon reconstitution) stability study and (v) photostability study.

The *Primary Stability Study* (ii) and *Reconstitution Stability Study* (iv) were performed in a (b) (4) design consisting of (b) (4) of 500 IU and 3000 IU DP presentations, representing the (b) (4) of DP strengths. (b) (4) 2000 IU was also included in the primary stability study. In the *Supportive Stability Studies* (i) (b) (4) lots of 500 IU and (b) (4) of 2000 IU were investigated. For the *PPQ Study* (iii), (b) (4) consecutive DP lots were used. The following DP lots and storage conditions were studied.

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

(b) (4)

The parameters tested were Appearance of Powder, Reconstitution Time/Solubility, (b) (4) Appearance of Solution, (b) (4), Protein Content, (b) (4), Purity, Potency, Particulate Matter, Sterility and Bacterial Endotoxins. Upon performing the studies, all parameters were within specifications. Additional details were the following.

1. In *Primary, Supportive* and *PPQ Stability* studies, during storage at 5°C and 30°C without preceding storage at 5°C, an increase in (b) (4) and slight decrease in Purity were observed upon the long-term storage at 30°C for 12 months, however, the parameters were within specifications limits.
2. In *Primary* and *Supportive Stability* studies, during storage at 30°C with and without preceding storage at 5°C, an increase in (b) (4), and decrease in Purity were observed upon the long-term storage at 5°C for 18 months followed by storage at 30°C for 12 months. All results for all PPQ batches stored for up to 12 months at 5°C and 30°C, and 6 months at (b) (4) met with the acceptance criteria.
3. In *In-Use Stability* study, all parameters were within specifications' limits for (b) (4) when stored at 5°C. For storage at 30°C, all parameters were within specification for (b) (4). The data support stability in-use for (b) (4) when stored at 5°C and for (b) (4) when stored at 30°C.
4. Upon performing *Photostability* study, Novo concluded that the primary container should be protected from light, and the DP stored in the secondary packaging material is stable towards light exposure.

The results supported shelf-life of DP for 30 months when stored at 5°C, where the DP may be kept at or below 30°C for a single period up to 12 months. Reconstituted DP (in-use) may be kept until use for 24 h at 5°C or for 4 h at ≤ 30°C. In addition, the DP Solvent, 0.9% Sodium Chloride, is stable for 60 months when stored at 5°C (b) (4) (section 3.2.P, Solvent).

3.2.P.8.2 Post-Approval Stability Protocol and Stability Commitment

Novo provided the following post-approval stability study protocol and commitments:

- 1) For long-term real-time stability study of PPQ lots, the study will continue until the (b) (4) months-time point.
- 2) For long-term real-time stability study:
 - (b) (4) of each dosage strength, 500 IU, 1000 IU, 1500 IU, 2000 IU and 3000 IU, will be placed in the long-term stability study (b) (4). If a particular strength is not manufactured in a particular year, this strength is exempted from the study. The test program is the same as that in the current primary and supportive stability study for long-term testing (storage at 5°C ± 3°C/ambient (b) (4) storage at 12 months at 30°C (b) (4).
 - After the (b) (4) of production, the selection of lots for the study will be based on a design where (b) (4) manufactured lots representing all five strengths of FDP will be placed in the on-going stability program (b) (4). The long-term test program for the storage at 5°C followed by storage at 30°C with reduced stability testing frequency (the time interval is testing at (b) (4) months when stored at 5°C, and (b) (4) months when stored at 30°C).
- 3) Novo Nordisk will submit the results of the stability studies in annual reports as specified by regulatory agencies.

Overall Reviewer's Assessment of Section 3.2.P.8:

As discussed in section 3.2.P.3.5, the reviewers requested the Applicant to provide more information to support the use of the (b) (4) approach in process validation. Novo submitted data with stability trend graphs for a completed "accelerated" stability study (STN 125671/57). The study was performed at storage conditions of (b) (4) on primary, supportive and PPQ lots, and a post-PPQ (b) (4) 1500 IU dosage strength (total of (b) (4) lots). The tested stability-indicating parameters were (b) (4), Protein Content, (b) (4), Purity and Potency. Except for two single results for (b) (4), obtained as the last time-point of the storage, comparable stability profiles were observed for all product dosage strengths. For the last time-point (6 months), two results for (b) (4) (500 IU and 1500 IU lots) were higher compared to the other lots. Increase of (b) (4) at the very last time point of the studies is expected for accelerated conditions of the storage and is not expected for the normal storage conditions. These results supported the (b) (4) approach and justified process validation for all dosage strengths including 1500 IU dosage strength.

In conclusion, the stability testing program was designed appropriately. All concerns were adequately addressed in the course of review. The study results justify and support the proposed shelf-life and storage conditions of DP: 30 months when stored at 5°C, where the DP may be kept at or below 30°C for a single period up to 12 months. Reconstituted DP (in-use) may be kept until use for 24 h at 5°C or for 4 h at ≤ 30°C.

3.2.P DRUG PRODUCT (Solvent)

The solvent (diluent) for lyophilized DP is 0.9% Sodium Chloride Solution. The Sodium Chloride is of (b) (4) which is produced by Novo Nordisk for all their parenteral products. The solvent (4 mL) is supplied in a pre-filled syringe (container closure system, 5 mL) made of siliconized borosilicate glass (b) (4) and a siliconized plunger made of bromobutyl rubber (b) (4). The syringe closure system has a tip cap with a luer lock and a tamper-evident seal. The tip cap is also made of bromobutyl rubber (b) (4). The pre-filled syringe is supplemented with vial adapter, which, altogether with container closure system for DP, are defined as a (b) (4).

The same solvent (b) (4) is supplied for NOVOEIGHT (STN 125466). The same (b) (4) lots of solvent (b) (4) were used for validation of the use in both ESPEROCT and NOVOEIGHT. These lots (b) (4) were produced on (b) (4), respectively, at (b) (4). Therefore, the solvent and (b) (4) used in ESPEROCT are considered suitable for the intended purpose.

Overall Reviewer's Assessment of Section 3.2.P (Solvent):

The vial with lyophilized DP, pre-filled syringe with diluent and vial adapter are packed together in ESPEROCT and constitute a drug (b) (4) which has been previously approved for several Novo's products approved for treatment of hemophilia including NOVOSEVEN (STN 103665), NOVOEIGHT (STN 125466) and REBINYN (STN 125611). Based on the submitted information, the (b) (4) is considered to be suitable for reconstitution of lyophilized ESPEROCT and use in patients.

3.2.A APPENDICES

3.2.A.1 Facilities and Equipment

This information was reviewed by OCBQ/DMPQ.

3.2.A.2 Adventitious Agents Safety Evaluation

Evaluation of safety regarding adventitious agents was performed for raw materials of biological origin. These materials are non-compendial and include (b) (4)

The potential of contamination of these materials with non-viral adventitious agents such as bacteria, fungi, and mycoplasma is well controlled by the following means.

- Appropriate environmental control monitoring during the manufacturing process.
- Validated cleaning/sanitization procedures in the manufacturing process.
- In-process controls, e.g., testing for microbial growth and mycoplasma in (b) (4).
- Filtration steps including (b) (4) sterile filtration. The potential of ESPEROCT to be contaminated with non-viral adventitious agents is further reduced by testing the final product for Sterility and Endotoxin. Novo manufactures the DP according to GMP regulations.

The risk of adventitious viruses or transmissible spongiform encephalopathy agents is minimized because there are no raw materials or ingredients of human or animal origin used in the manufacturing process. The evaluation of safety regarding virus contamination of the biological raw materials and overall manufacturing process capacity in virus clearance is reviewed below.

(b) (4)

(b) (4)

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

(b) (4)

Capacity of the ESPEROCT purification process to clear viruses

There are two dedicated, (b) (4) steps for viral clearance in the manufacturing process, (b) (4) Triton X-100 (b) (4) and Nanofiltration (b) (4). The Purification by (b) (4) step in the manufacturing process also contributes to virus removal. The viruses selected for the studies include (b) (4)

These viruses have a wide range of physico-chemical properties covering those of viruses which may contaminate ESPEROCT, by this representing an adequate model to verify the ability of the manufacturing process to eliminate viruses. Virus clearance studies were performed by spiking the viruses at defined amounts into samples collected at various manufacturing steps and analyzing those in processed samples. (b) (4).

Reviewer's comment

To evaluate the sufficiency of the viral clearance in each study, each down-scale system used needed to be qualified, whereas such data were incomplete in the original BLA. Therefore, the reviewer asked Novo to provide: (i) data to demonstrate that each down-scale system used for viral clearance studies was representative to (b) (4) and (ii) data on cytotoxicity to demonstrate that the components used in the assays do not adversely affect the (b) (4) in relevant viral clearance studies. In response (STN 125671/25), the company provided information supporting virus clearance studies for the following steps.

1) (b) (4) Triton X-100 (b) (4)

The comparability of the down-scale and the manufacturing-scale was demonstrated by (b) (4)

The viral clearance data derived from the down-scale system are appropriate to be used for evaluating the viral clearance capacity of this step at manufacturing-scale. The acceptance criteria of the critical parameters of this step in the down-scale studies on (b) (4) are listed below.

(b) (4)

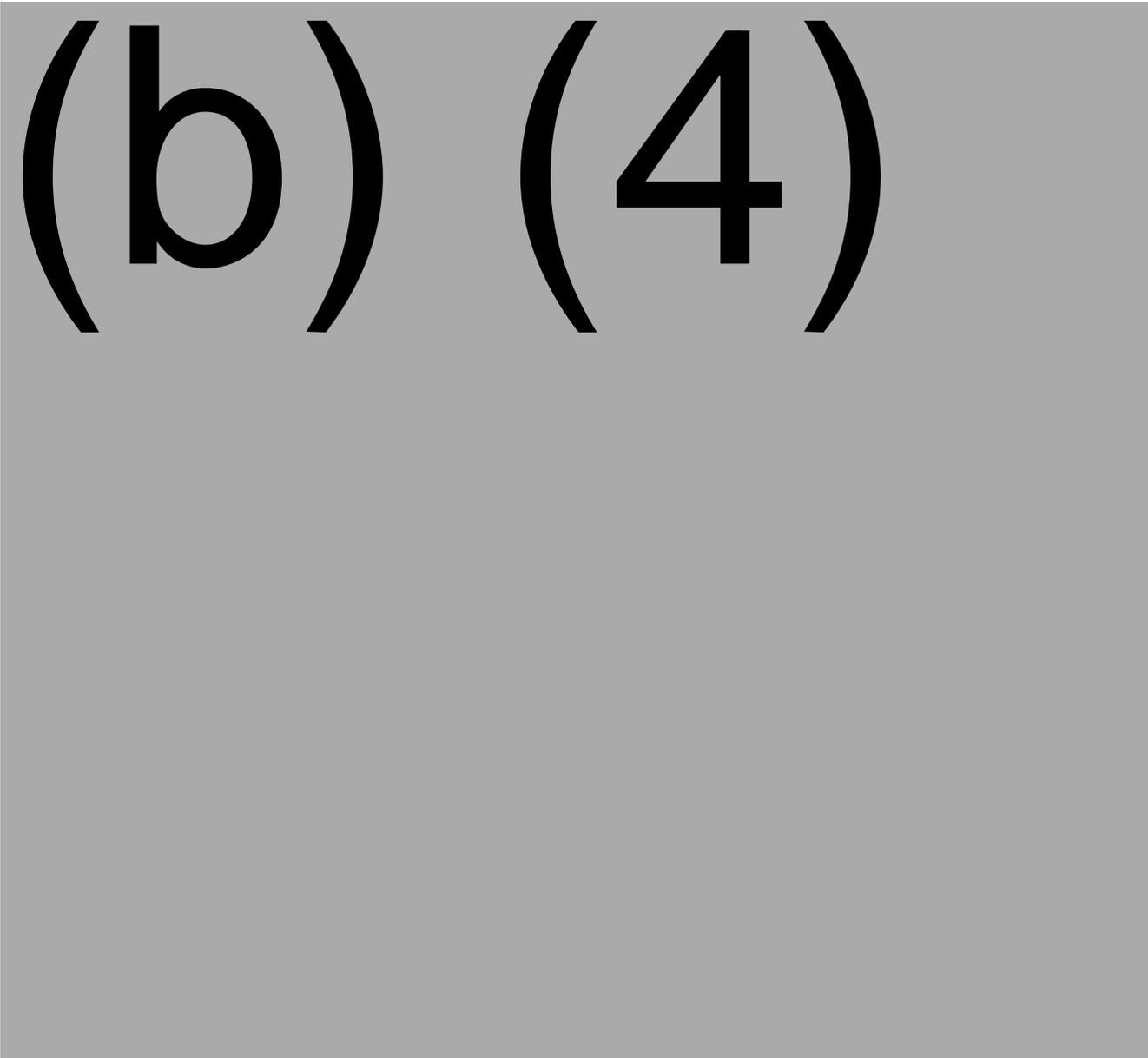
Also, Novo provided additional data on cytotoxicity and interference to show that the components used in virus (b) (4) assays do not adversely affect the (b) (4) in the viral clearance studies. The data from the GLP study reports 215043 and 300075 are summarized on the following table. These results show that the infectivity was below the LOD for (b) (4). Thus, the viral reduction factors for (b) (4) are estimated to (b) (4).

(b) (4)

(b) (4)

(b) (4)

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



Reviewer's comment

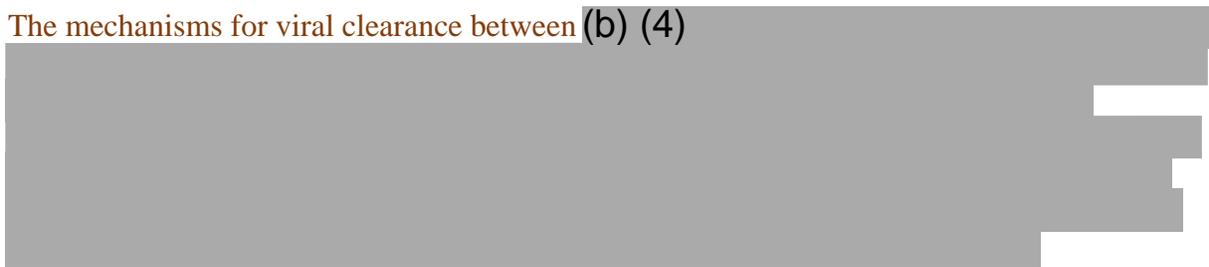
(b) (4)

Therefore, the viral clearance data presented on the above table are acceptable.

Virus selection in the down-scale studies is consistent with the FDA recommendation regarding the biological drug products derived from cell lines of human or animal origin. The qualification of the down-scale systems used for viral clearance is acceptable, and the viral

clearance data derived from these down-scale systems are sufficient to support the effectiveness of viral clearance in the proposed commercial manufacturing process. Thus, the provided information is acceptable.

The mechanisms for viral clearance between (b) (4)



Overall Reviewer's Assessment of Section 3.2.A.2:

The potential contamination of raw materials of biological origin by bacteria, fungi, and mycoplasma is well controlled. The risk of transmissible spongiform encephalopathy agents is minimized because there are no raw materials or ingredients of human or animal origin used in the manufacturing process. Performed studies support high efficiency of the manufacturing process to clear viruses that can potentially contaminate drug product. Altogether, provided information supports safety of ESPEROCT regarding adventitious agents. Thus, the information provided in this section is acceptable.

3.2.A.3 Novel Excipients

No new excipients are used in production of ESPEROCT.

3.2.R Regional Information (USA)

Executed Batch Records

The master batch records are provided for DS, DP and Solvent.

1. For DS, the executed batch records were provided for ^{(b) (4)} PPQ batches (b) (4) in respective reports as follows.
2. For DP, the executed batch records were provided for ^{(b) (4)} PPQ lots with dosage strengths of 500, 1000, 2000 and 3000 IU (b) (4), respectively).
3. For Solvent (0.9% Sodium Chloride Solution), the executed batch records were provided for a PPQ lot in non-translated report.

(b) (4)

Method Validation Package

This section contains methods validation package (document #003797975) which includes description and reports for validation of the following analytical procedures, discussed in sections 3.2.S.4.2-3 and 3.2.P.5.2-3.

Table 3.2.R-1. (b) (4) Procedures Used for ESPEROCT Analysis

Test parameter	Pharmacopeial procedure	Pharmacopoeia	Pharmacopoeia procedure ID	Documentation
(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Appearance of solution	Visual evaluation of clarity	(b) (4)	(b) (4)	NA
	Colour			NA
	Particulate contamination/ Foreign insoluble matter			NA
(b) (4)	(b) (4) y	(b) (4)	(b) (4)	(b) (4)
Particulate matter	Particulate matter			(b) (4)
	(b) (4)			(b) (4)
(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Bacterial endotoxin	Bacterial endotoxins (b) (4)			(b) (4) - Bacterial Endotoxins -
Sterility	Sterility	(b) (4)	(b) (4)	(b) (4)
	(b) (4)			- Sterility by (b) (4)

Table 3.2.R-2. Non-pharmacopeia Procedures Used for ESPEROCT Analysis

Test parameter	Analytical procedure no.	Documentation
Identity (b) (4) and Purity	(b) (4)	(b) (4) Identity and Purity by (b) (4) (b) (4) Identity and Purity by (b) (4)
Protein content (b) (4)	(b) (4)	(b) (4) (b) (4)
Potency	(b) (4)	(b) (4) Potency by Chromogenic Assay (b) (4) Potency by Chromogenic Assay
Appearance of powder and Reconstitution time/solubility	(b) (4)	(b) (4) Appearance of Powder and Reconstitution Time (b) (4) Appearance of Powder and Reconstitution Time
(b) (4)	(b) (4)	(b) (4)
Polysorbate 80	(b) (4)	(b) (4) Polysorbate 80 (b) (4)
Sucrose	(b) (4)	(b) (4) Sucrose (b) (4) Sucrose (b) (4)
(b) (4)	(b) (4)	(b) (4)
Calcium	(b) (4)	(b) (4)

Combination Products

1. The document #001223363 contains description of (b) (4) vial adapter conformity to standards and the unit drawing. The adapter is manufactured by (b) (4) (b) (4) and referenced as 510(K) (b) (4). It is made of polycarbonate, and stated to not contain phthalates, materials of human or animal origin, latex components and conflict minerals. The section also contains a letter to authorize FDA to refer to the 510(k) of the vial adapter and to the following.

- DMF (b) (4), Lyophilization Stopper 13 mm grey ((b) (4)
- DMF (b) (4), Syringe barrel 5 mL (b) (4)
- DMF (b) (4), Rubber plunger 5 mL (b) (4)
- DMF (b) (4), Syringe closure system 5 mL (b) (4)

2. The document #003650898 contains summary of assessment of compatibility of the vial adapter with reconstituted DP. The vial adapter is a sterile, disposable device. The adapter allows for transfer of fluids into and out of vials. The solvent is transferred from the syringe into the vial containing the lyophilized powder. Next, the reconstituted DP is transferred from the vial back into the syringe. The vial adapter has a (b) (4) in-line filter which allows particulate filtration and flow aspiration. The vial adapter was justified for its intended use with regard to sorption, precipitation, discoloration, stability, extractables, leachables and safety.

In *Compatibility* study, the DP formulations with the highest and lowest content of ESPEROCT (3000 IU and 500 IU) were tested. The reconstituted solutions were tested for Appearance, (b) (4), Protein Content, (b) (4), Potency, Purity, (b) (4). All parameters stayed within the pre-defined acceptance criteria and no precipitation or discoloration was detected.

Extractables study used (b) (4)

No leachable compounds from the adapter were detected at levels representing a toxicological concern. Based on all results, it was concluded that the adapter is compatible with ESPEROCT and suitable for its intended use.

Overall Reviewer's Assessment of Combination Products Section:

No deficiencies were identified. The information provided is acceptable as submitted.

Comparability Protocols

No comparability protocols for future planned changes were provided. No changes were made in the manufacturing process, facilities and equipment between DP lots used in the Phase 3 clinical studies and conformance lots.

Other eCTD Modules

Module 1

1.12.14 Environmental Assessment or Claim of Categorical Exclusion

The BLA included a request for categorical exclusion from an Environmental Assessment under 21 CFR 25.31(c). The FDA concluded that this request is justified as the manufacturing of this product does not alter significantly the concentration and distribution of naturally occurring substances, and no extraordinary circumstances exist that would require an environmental assessment. This information was reviewed in by OCBQ/DMPQ.

1.14 Labeling

The Full Prescribing Information (FPI) and the product package and container labels were reviewed, commented, and revised by the appropriate discipline reviewers and by the APLB from a promotional and comprehension perspective during the labeling negotiations (December 2018 – February 2019).

Full Prescribing Information (FPI):

The CMC reviewers reviewed and revised the following sections of the FPI:

Dosage Forms and Strengths (3):

“ESPEROCT is available in single-dose vials containing nominally 500, 1000, 1500, 2000 or 3000 IU, after reconstitution, containing approximately 125, 250, 375, 500 or 750 IU per mL, respectively. The actual FVIII activity is printed on each ESPEROCT vial and carton.”

Monitoring Laboratory Tests (5.3):

“If monitoring of Factor VIII is performed, use a chromogenic or one-stage clotting assay appropriate for use with ESPEROCT [*see Dosage and Administration (2)*].

Factor VIII activity levels can be affected by the type of activated partial thromboplastin time (aPTT) reagent used in the assay. Some silica-based aPTT reagents can underestimate the activity of ESPEROCT by up to 60%; other reagents may overestimate the activity by 20%. If an appropriate one-stage clotting or chromogenic assay is not available locally, then use a reference laboratory.

If bleeding is not controlled with the recommended dose of ESPEROCT or if the expected Factor VIII activity levels in plasma are not attained, then perform a Bethesda assay to determine if Factor VIII inhibitors are present.”

Description (11):

ESPEROCT is described as a sterile lyophilized powder for intravenous injection after reconstitution. The active ingredient is a recombinant BDD-FVIII produced in CHO cells, purified and conjugated with 40-kDa PEG molecule via the O-glycan. A concise summary of the manufacturing process is presented. The excipients used in the formulation are listed. Activation process of rFVIII-PEG is briefly discussed.

Clinical Pharmacology (12):

This section includes description of mechanism of action of ESPEROCT, and its pharmacodynamics and pharmacokinetics. Upon administration of the dosage of 50 IU/kg into patients ≥ 12 years, the half-life of rFVIII-PEG in the circulation was (b) (4)

How Supplied/Storage and Handling (16):

This section provides description of the kit components, and storage and handling instructions for lyophilized and reconstituted product.

The final version of FPI submitted on February 12, 2019 was determined to be acceptable.

Carton and Container Label:

In section 1.14.1.1, the primary container (vial) label states: nominal and actual potency per vial (IU), storage conditions, reconstitution solution name (sodium chloride), contact phone number of Novo, expiration date and lot number. The secondary container (carton) label contains the same information, and in addition, serial number, stability upon reconstitution data, list of excipients, U.S. License number, the directions: “Intravenous use, after reconstitution. Single dose. Discard unused portion” and description of the enclosed parts as “Includes (b) (4) vial adapter and a pre-filled diluent syringe”. For the Solvent, the label contains description of its volume amount (4 mL), intended use, storage condition, bar code number, Novo’s contact phone number, expiry date and lot number. All labels were found acceptable.

Modules 4 and 5

ANALYTICAL PROCEDURES AND VALIDATION OF ANALYTICAL PROCEDURES FOR ASSESSMENT OF CLINICAL AND ANIMAL STUDY ENDPOINTS

4.2.2.2.1 Analytical methods and validation reports (non-clinical studies)

This section contains studies reports for validation of the following analytical methods.

- *Validation of (b) (4) (NNC 0129-0000-1003) antibody (b) (4) for use in (b) (4) s and Rat citrate plasma (study report #209384).* The method validation was focused on assay sensitivity, recovery and interference from free drug and hemolysis, assay precision, drift, and specificity. The study was performed according to recommendations of *Mire-Sluis Barrett et al, 2004* [1] and *Shankar et al, 2008* [2].
- *Validation of a FVIII Clotting Activity Assay for Quantification of N8 GP Activity in Citrated (b) (4) Monkey Plasma (study report # 209407).* The study was focused on assessment of the assay performance, i.e. validation and defining the acceptance criteria of an FVIII clotting activity assay in citrated monkey plasma containing relevant levels of rFVIII-PEG. The assay parameters were validated in accordance with an FDA guideline [3], an internal SOP #053698 (ed. 4.0), *DeSilva et al (2003)* [4] and *Viswanathan et al. (2007)* [5].

- *Validation of a FVIII Clotting Activity Assay for Quantification of N8-GP Activity in Citrated Rat Plasma* (study report # 209408). The study was focused on assessment of the same assay parameters in citrated rat plasma containing relevant levels of rFVIII-PEG. The assay/parameters were validated in accordance to the references [3-5].
- *Validation of a N8-GP neutralizing antibody assay in RAT plasma* (study report # 209489). The study was aimed to validate a neutralizing activity assay for the assessment of the neutralizing capacity of anti-rFVIII-PEG antibodies in rat citrate plasma. The assay is performed according to SOP # 125859, *Solberg 2010* [6] and *Harlow et al, 1988* [7].

Validation of the above methods was based on ICH Q2 (R1) *Validation of analytical procedures: text and methodology* and confirmed (determined) the respective ranges, accuracy, precision, sensitivity, specificity, drift, recovery. Section 4.2.2.2.1 was also reviewed by OCBQ/DBSQC.

References

1. Mire-Sluis Barrett, Y.C. et al, 2004. Recommendations for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products. *Journal of Immunological Methods* 289, 1- 16.
2. Shankar, G., et al, 2008. Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products. *Journal of Pharmaceutical and Biomedical Analysis* 48 (2008) 1267–1281.
3. U.S. Department of Health and Human Services. FDA/CDER/ Center for Veterinary Medicine (CVM). May 2001. BP: Guidance for Industry. *Bioanalytical Method Validation*.
4. DeSilva B. et al, 2003, Recommendation for the Bioanalytical Method Validation of Ligand-binding assays to support Pharmacokinetic assessments of Macromolecules. *Pharm. Res.* 2003; 20: 1885-1900.
5. Viswanathan CT. et al, 2007, Quantitative bioanalytical methods validation and implementation: best practice for chromatographic and ligand binding assays. *The AAPS Journal* 2007; 9: E30-E42.
6. Solberg, H. 2010. Long time stability of human antibodies in serum stored at minus 20°C.
7. Ed Harlow and David Lane: *Antibodies; A laboratory Manual*. Cold Spring Harbor Laboratory 1988, pp 119, 285, 287, 291.

5.3.1.4 Reports of bioanalytical and analytical methods for human studies

This section contains study reports for validation of the following analytical methods used in clinical studies.

1. Validation of the FVIII Bethesda Assay (b) (4) for the Detection and Quantification of FVIII inhibitors in human plasma

The report describes the analytical validation of the so-called (b) (4) of the Bethesda assay for quantification of inhibitory antibodies directed against FVIII. This assay is routinely used to detect and quantify inhibitory antibodies against FVIII in the patients treated with replacement FVIII products. Validation was performed in 2008 and final report was issued

on April 17, 2009. Novo stated that at the time of validation study standardized procedure, validation report or a detailed and approved protocol for the (b) (4) of Bethesda assay has not been published yet. Novo also stated that due to the nature and intended use of the assay they were not able to follow strictly the Guidance for Industry, Bioanalytical Method Validation issued by the FDA, which is acceptable. The study showed that the assay described was suitable for the measurement of FVIII inhibitors in human plasma. Following robustness parameters were investigated and identified during the validation:

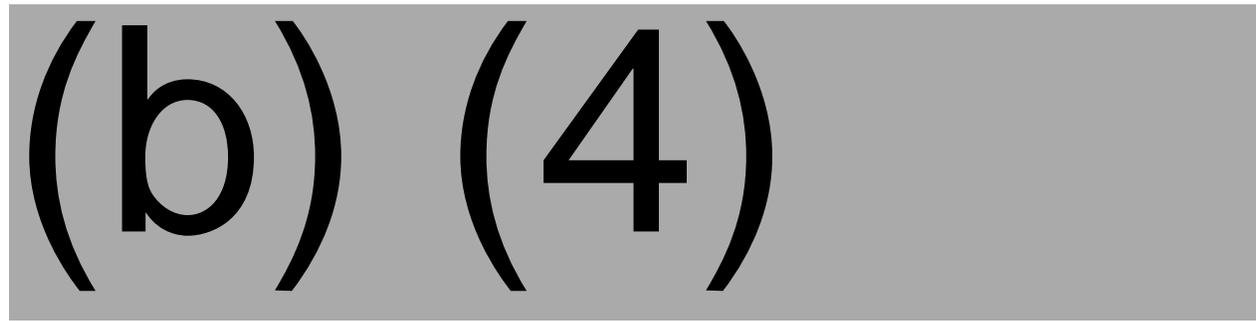
- (b) (4)

2. Supplementary Validation of the FVIII Bethesda Assay (b) (4) for the Detection and Quantification of FVIII inhibitors in human plasma

The report described supplemental validation study performed for (b) (4) of the Bethesda assay, described above, in 2010 (report finalized November 12, 2010). The scope of the study was limited to determine the sensitivity of the assay to interference from ESPEROCT (previously non-PEGylated recombinant FVIII was used). It was shown that up to (b) (4) of recombinant FVIII can be tolerated in the assay and still detect low level inhibitors.

3. Validation of anti-rFVIII-PEG antibody (b) (4) in Human Plasma.

The report describes the analytical validation of (b) (4) for quantification of (b) (4). The method is different from the Bethesda assay in that all (b) (4). Novo used a standard (b) (4), involving (b) (4). The study was performed in 2010 (report finalized May 27, 2011). Results of the validation are presented in the following table.



(b) (4)

The study may not be called validation study, as it did not include prospectively determined acceptance criteria, which were established retrospectively. However, the results demonstrate that assay is suitable to its intended purpose. The studies performed allowed to determine method capabilities and overall package is acceptable, considering the (b) (4) nature of this method and the fact, that method was prospectively validated upon transfer to the site where analysis was performed for clinical samples (see below).

4. Cross-site validation of the (b) (4) method for the (b) (4) determination of rFVIII-PEG antibodies in human citrate plasma samples

The purpose of this study was to validate the method transfer of (b) (4) method described above from Novo to (b) (4). The study was designed based on the data obtained from initial validation performed at Novo, and performed in 2011 (report finalized August 29, 2012). All reported results fulfilled the acceptance criteria demonstrated that the method is suitable for the determination of anti-rFVIII-PEG antibodies in human citrate plasma. While multiple parameters were tested, and the data are too voluminous to put in the review memo, the key performance parameter is a (b) (4)

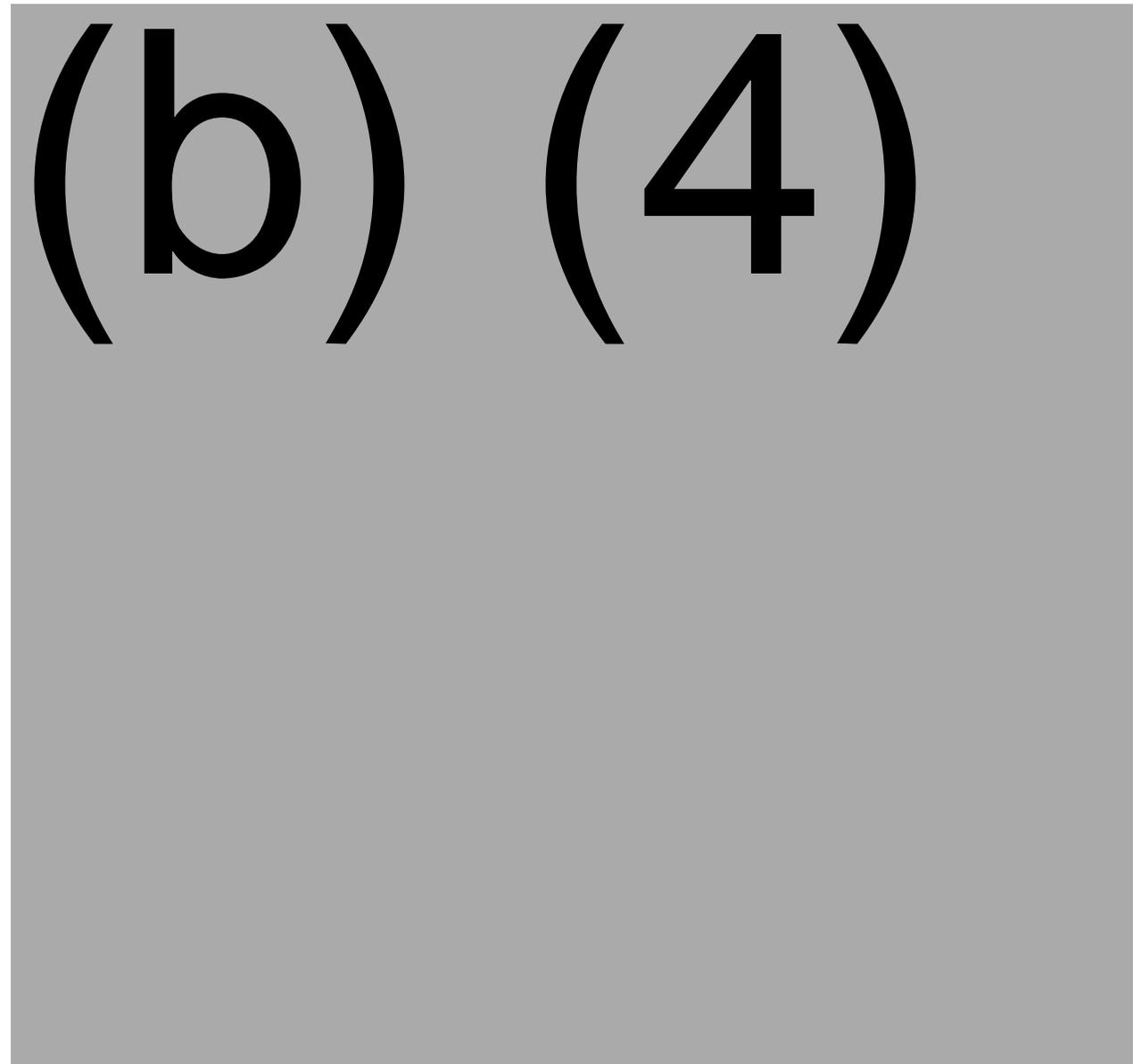
(b) (4) which is acceptable for such method.

5. Validation of a rFVIII-PEG (b) (4)

In this study, an (b) (4) method was validated for detection of (b) (4) antibodies against rFVIII-PEG in human citrate plasma. (b) (4) antibodies are indicative of allergic reaction. The study was performed in 2013 (report finalized September 17, 2015). (b) (4) is proprietary (b) (4) and routinely used to detect allergic response by detecting IgE antibodies to various substances. The method uses (b) (4)



Results of the validation are presented in the following table.



(b) (4)

(b) (4)

6. Validation of an (b) (4) method for the detection of PEG antibodies in human plasma (citrate)

The purpose of this study was to validate an (b) (4) method for the determination of antibodies against polyethylene glycol (PEG) in citrated human plasma. As rFVIII-PEG is a PEGylated protein, Novo developed assay to monitor patients for potential antibodies directed against the PEG moiety in rFVIII-PEG. The study was performed in 2014 (report finalized December 18, 2015) at (b) (4). The results are presented in the following table.

(b) (4)

(b) (4)

The study included limited number of predefined acceptance criteria, including for the CV% of replicates and requirements for CV% for cut point and precision runs. For remaining parameters, the acceptance criteria were established based on a study to determine the performance of cut point and precision runs and were applied to any subsequent run. The results demonstrated that assay is suitable to its intended purpose.

7. Validation of an (b) (4) assay for the determination of anti-CHO-HCP antibodies in human sodium citrate plasma.

This study was performed to validate an (b) (4) method for the determination of antibodies against CHO HCP in citrated human plasma. The purpose of the assay was to monitor for a potential immune response against HCP impurities in ESPEROCT. The study was performed in 2017 (report finalized August 30, 2017) at (b) (4). The results of are presented in the following table.

(b) (4)

(b) (4)

(b) (4)

8. Validation of FVIII activity assays used in clinical trials (pharmacokinetics assays)

Novo provided the following assays qualification reports:

- 210316. *Validation of a FVIII chromogenic activity assay for N8-GP in human citrate plasma using N8-GP calibration*
- 210317. *Validation of a FVIII clotting activity assay for N8-GP in human citrate plasma*
- 213071. *Validation of FVIII activity stability at (b) (4) for N8-GP in human citrate plasma*
- 300122. *Validation of the (b) (4) FVIII (N8-GP) chromogenic assay*
- 212108. *Incurred sample reproducibility of FVIII activity analysis with a chromogenic assay (calibration with N8-GP) of samples from trial NN7088-3776*
- 212109. *Incurred sample reproducibility of FVIII activity analysis with a clotting assay (calibration with N8-GP) of samples from trial NN7088-3776*

In the studies, both clotting and chromogenic FVIII assays were used. In most cases, Novo used assays calibration using a product-specific reference standard rFVIII-PEG. Appropriate assay controls were used in each run of the assay and in all assay qualifications studies. The assays, calibrated using normal pooled plasma, were also used in most recent studies. All assays were properly bridged to each other using the rFVIII-PEG and (b) (4) standards.

Reviewer's comments

1) Although the use of product-specific standards for calibration of FVIII activity assays is preferred from the analytical perspective, such standards are not available for routine use by clinical laboratories. These assays are always calibrated using a FVIII standard prepared from pooled normal plasma. Novo stated that they were not able to develop a robust clotting assay

calibrated using normal pooled plasma, therefore, they outsourced the development of this assay to a well-known specialized hemostasis clinical laboratory. The difficulties experienced by Novo's clinical labs in calibration of the clotting assay for rFVIII-PEG activity suggest that the clinical labs will have similar problems when testing plasma of ESPEROCT-treated patients. Therefore, it would be important for Novo to provide assistance to interested clinical labs with qualification of their routine assays. For example, Novo may want to share their reagents and rFVIII-PEG samples with such laboratories.

2) All clinical FVIII activity assays were additionally qualified using a set of hemophilia plasma samples spiked with several licensed FVIII products, providing evidence that Novo's assay performance is consistent with the assays used in routine clinical labs.

9. Clinical lab field study to investigate factor activity assay discrepancies

Accurate determination of circulating FVIII activity levels is important to patients' care as underestimation or overestimation of those may lead to inaccurate dosing. The purpose of this study was to determine the consistency of FVIII activity measurements and to clarify which reagents, standards, equipment *etc* are more suitable for rFVIII-PEG analysis. For that purpose, hemophilia A plasma samples were spiked with either rFVIII-PEG or full-length rFVIII product, (b) (4), at various concentrations (b) (4). A normal plasma standard with an assigned FVIII value of (b) (4) was used as control.

The samples were blinded and sent to clinical laboratories worldwide that analyzed the samples using their routine FVIII activity assays to determine FVIII activity in the samples. A total of 67 laboratories from 25 countries participated in this study; 60 laboratories used the one-stage clotting assay and 36 laboratories used the chromogenic assay for FVIII measurements. (b) (4) different aPTT reagents and (b) (4) different chromogenic kits were used. The study results were the following.

a) (b) (4)

Taken together, the results showed that rFVIII-PEG can be measured in plasma using both conventional FVIII assays (one-stage clotting and chromogenic). However, the results may be overestimated or underestimated (vs. the "actual" spiked rFVIII-PEG activity) if clinical assays calibrated using a normal pooled plasma reference standard rather than a product-specific FVIII

activity calibrator. For the one-stage clotting assay, some (b) (4) reagents should be avoided as they cause substantial underestimation.

Reviewer's comment

The study design is consistent with the FDA recommendations provided to manufacturers of extended half-life factor products. The results of several similar investigations were published in recent years. Comments for the Novo's conclusions are the following.

- a) Using different aPTT reagents may result in underestimation of rFVIII-PEG activity by (b) (4) 60%, or by about 20%. This comment was included in the Package Insert for ESPEROCT (section 5.3 *Monitoring Laboratory Tests*).
- b) Overestimation of (b) (4) activity by clotting assays may indicate incorrect potency assignment of (b) (4) vials used by Novo. Indeed, Novo acknowledged such uncertainty in the assignment of potency values.
- c) From results, the obvious conclusion should be that all chromogenic assays kits overestimated FVIII activity for both rFVIII-PEG (b) (4). The kit qualified as showing the lowest recovery, actually provided almost target values of FVIII activity.
- d) Underestimation of FVIII activity in patients' plasma by clotting assays may result in overdosing patients and thrombotic conditions, whereas overestimation the activity by chromogenic assays may result in under-dosing and risk of bleeds. In addition, underestimation of a factor activity in plasma samples carries higher risk for extended half-life products like rFVIII-PEG.

Overall Reviewer's Assessment of Relevant Sections of Module 4 and 5:

Methods used in non-clinical and clinical studies were demonstrated to be suitable for their intended purpose. No deficiencies were identified. While some validation studies did not follow standard validation approach, including absent or incomplete pre-defined acceptance criteria, they were still sufficient to demonstrate the methods' suitability and measure performance parameters. Considering semi-quantitative nature of the methods, the reviewers do not consider this to be an issue. The potential under-estimation or over-estimation of FVIII activity in post-infusion plasma samples is adequately reflected in the FPI, section 5.3. Thus, the information on validation of analytical methods in sections 4.2.2.2.1 and 5.3.1.4 is acceptable.