

CBER CMC BLA Review Memorandum

BLA STN 125690

**ERVEBO [Ebola Zaire Vaccine, Live; for intramuscular
administration only; (≥ 72 million pfu/1 mL dose)]**

**Dmitriy Volokhov, DVM, PhD
Staff Scientist/Research Microbiologist
CBER/OVRR/DVP/LMD**

1. BLA# STN 125690

2. APPLICANT NAME AND LICENSE NUMBER

Merck Sharp & Dohme Corp.

3. PRODUCT NAME/PRODUCT TYPE

Ebola Zaire Vaccine, Live (tradename: ERVEBO); is a vaccine for intramuscular administration containing ≥ 72 million pfu/1 mL dose.

4. GENERAL DESCRIPTION OF THE FINAL PRODUCT

ERVEBO is a live attenuated Ebola Zaire (rVSV Δ G-ZEBOV-GP) recombinant viral monovalent vaccine consisting of a vesicular stomatitis virus (VSV) backbone deleted for the VSV-G envelope glycoprotein and substituted with the envelope glycoprotein (GP) of the *Zaire ebolavirus* (Kikwit 1995 strain). The vaccine is manufactured in serum-free Vero cell cultures. The virus is harvested from the cell culture medium, purified, and (b) (4). (b) (4), diluted as needed, mixed, filled into vials, inspected, labeled, packaged, frozen, and stored at -80°C to -60°C. Each 1.0 mL dose of vaccine contains a minimum of 72 million plaque forming units (pfu) of rVSV Δ G-ZEBOV-GP live virus in a stabilizer solution consisting of 10 mM Tris, 2.5 mg/mL rice-derived recombinant human serum albumin (rd-rHSA) and water for injection. The vaccine may contain trace amounts of rice. The vaccine should appear as a colorless to slightly brownish-yellow liquid with no particulates visible. The vaccine contains no adjuvant or preservatives. The vaccine is a sterile solution for intramuscular injection and should be used directly as supplied. No dilution or reconstitution is necessary. ERVEBO is a vaccine indicated for the prevention of disease caused by *Zaire ebolavirus* in individuals 18 years of age and older.

5. MAJOR MILESTONES

Submission Date: 07/15/2019

Date of Filing Meeting: 08/27/2019

Filing Date: 09/13/2019

BLA Action Due Date: 03/14/2020

6. CMC/QUALITY REVIEW TEAM

Affiliation	Reviewer	Section/Subject Matter
CMC Reviewer	Dmitriy Volokhov, DVM, PhD	Sections 2.2, 2.3, 2.4, 3.2.S, 3.2.P, 3.2.A.2, 3.2.A3, 3.2.R, 4.2.1, and 5.3.1
CMC Reviewer (metagenomics)	Arifa Khan, PhD	Adventitious Agents safety evaluation and validation of viral clearance (Sections S.2.3, S.3.2., P.4.5, P.5.5 and A.2)
CMC Inspector	Christian Sauder, PhD	The pre-approval inspection at (b) (4); Establishment Inspection Report)
OCBQ/DMPQ – Lead Inspector and Team Lead	Christian Lynch	The pre-approval inspection at (b) (4); Establishment Inspection Report)
OCBQ/DMPQ – Reviewer and Inspector	Richard Lewis, PhD	The pre-approval inspection at (b) (4), 2019; Establishment Inspection Report). Sections 3.2.S.2.1, 3.2.S.2.2, 3.2.S.2.4, 3.2.S.2.5, 3.2.S.6, 3.2.P.2.4, 3.2.P.3, 3.2.P.7, 3.2.A, and 3.2.A.1.
Statistical Reviewer - assays	Charles Cheung, PhD	Module 1.11.3 Clinical Assays
OCBQ/DBSQC Reviewer	Simleen Kaur, PhD	Section 3.2.S.4- Sterility and Mycoplasma Section 3.2.P.5- Sterility and Endotoxin

OCBQ/DBSQC Reviewer	Anil Choudhary, PhD, MBA	Validation results for Potency and Identity assays and SOPs for these assays. Sections 3.2.S.4.2, 3.2.S.4.3; Section: 1.11.1 (SOPs for Identity and Potency assays)
OCBQ/DBSQC Reviewer	Marie Anderson, PhD	Amendments 4, 22, and 29; LRP templates
OCBQ/DBSQC Reviewer	Ritu Agarwal, PhD	Validation results for Total Protein and (b) (4) assays and SOPs for these assays. Sections 3.2.S.4.2, 3.2.S.4.3, 3.2.P.5.2, 3.2.P.5.3.
Labeling – Carton, Container	Daphne Stewart	Section 1.14.1 Draft Labeling

7. SUBMISSION(S) REVIEWED

Date Received	Submission	Comments/Status
10/31/2018	STN 125690/0	Reviewed
12/13/2018	STN 125690/01 (a proposal to submit the Clinical modules with the Chemistry, Manufacturing and Controls (CMC) modules)	Reviewed
02/04/2019	STN 125690/04 (the lot release protocol templates)	Reviewed
02/22/2019	STN 125690/07 (response to IR dated February 13, 2019)	Reviewed
04/09/2019	STN 125690/15 (response to IR dated March 19, 2019)	Reviewed
05/01/2019	STN 125690/16 (response to IR dated April 10, 2019)	Reviewed
05/03/2019	STN 125690/17 (response to IR dated April 19, 2019)	Reviewed
07/11/2019	STN 125690/20 (response to IR dated July 2, 2019)	Reviewed
07/15/2019	STN 125690/21 (the interim (b) (4) PPQ and Comparability Reports which include data from (b) (4) PPQ Lots (b) (4); the Drug Product Container Closure Integrity Final report and updated stability data)	Reviewed
08/02/2019	STN 125690/25 (response to IR dated July 2, 2019)	Reviewed
08/27/2019	STN 125690/27 (an interim report for Drug Product Process Performance Qualification Lot Number (b) (4) (DP PPQ (b) (4)))	Reviewed
08/28/2019	STN 125690/29 (the revised lot release protocol templates)	Reviewed
09/10/2019	STN 125690/34 (an update to CBER on progress made on the Quality Improvement Plan at the MSD (b) (4) manufacturing site)	Reviewed
09/20/2019	STN 125690/35 (response to IR dated September 6, 2019)	Reviewed
09/25/2019	STN 125690/36 (the final report for an investigation into potency and benzonase results for (b) (4) Process Performance Qualification at the (b) (4) manufacturing site)	Reviewed
10/03/2019	STN 125690/38 (the interim results for (b) (4) PPQ Lot (b) (4), Final Report for Drug Product Process Simulation, and Final Report for Upstream Process Simulation Requalification)	Reviewed
10/14/2019	STN 125690/40 (the reports for (b) (4) Process Performance Qualification and Comparability)	Reviewed
11/01/2019	STN 125690/44 (the Final (b) (4) Process Performance Qualification Report including the (b) (4) and in vivo results)	Reviewed
12/02/2019	STN 125690/51 (responses to IRs dated November 21 and 25, 2019)	Reviewed
12/04/2019	STN 125690/52 (responses to IRs dated November 27 and 29, 2019)	Reviewed
12/11/2019	STN 125690/55 (responses to IRs dated December 6, 2019)	Reviewed
12/12/2019	STN 125690/56 (responses to IRs dated December 10, 2019)	Reviewed

8. REFERENCED REGULATORY SUBMISSIONS (e.g., IND, BLA, 510K, MASTER FILE, etc.)

Submission Type & #	Holder	Referenced Item	Letter of Cross-Reference	Comments/Status
IND 16131	Merck Sharp & Dohme Corp.	The original IND for this vaccine	no	Information was reviewed, assessed and documented.
MF (b) (4)	(b) (4)	Pharmaceutical Closure (Stopper)	yes	Information was reviewed, assessed and documented.

Submission Type & #	Holder	Referenced Item	Letter of Cross-Reference	Comments/Status
MF (b) (4)	Department of The Army, USA	Procedures and Validation Protocol for Ebola virus (Zaire) IgG Enzyme Linked Immunosorbent Assay (ELISA)	yes	Information was reviewed, assessed and documented.
MF (b) (4)	(b) (4)	Virus Production Serum Free Media (VP-SFM), (b) (4)	yes	This MF was originally submitted to CDER but was never reviewed there. Information on these materials provided in this BLA is sufficient.
MF (b) (4)	(b) (4)	(b) (4) Glass Vials	yes	This MF was originally submitted to CDER but was never reviewed there. Information on these materials provided in this BLA is sufficient.
MF (b) (4)	(b) (4)	Assay SOPs and Validation Reports	yes	Information was reviewed, assessed and documented.

9. REVIEWER SUMMARY AND RECOMMENDATION

A. EXECUTIVE SUMMARY

Merck submitted this BLA seeking approval of Ebola Zaire Vaccine, Live (tradename: ERVEBO) also referred to as rVSVΔG-ZEBOV-GP or V920 in this memo. I have reviewed the CMC section and preclinical studies. ERVEBO is a live attenuated Ebola Zaire recombinant viral monovalent vaccine consisting of a vesicular stomatitis virus (VSV) backbone deleted for the VSV-G envelope glycoprotein and substituted with the envelope glycoprotein (GP) of the *Zaire ebolavirus* (Kikwit 1995 strain) (rVSVΔG-ZEBOV-GP). The vaccine virus was constructed via recombinant DNA technology. The vaccine virus is propagated in serum-free Vero cell cultures, harvested from the supernatant and purified by (b) (4). The purified (b) (4) virus is produced under aseptic conditions and not subjected to further (b) (4). The final product is a mixture of the purified virus diluted in a stabilizer solution and filled into single dose vials and (b) (4). ERVEBO is a sterile solution for intramuscular injection. A single dose is 1.0 mL. Each 1.0 mL dose of vaccine contains a minimum of 72 million plaque forming units (pfu) of rVSVΔG-ZEBOV-GP live virus.

The (b) (4) used in the production of the vaccine were qualified for (b) (4). The sponsor presented information ensuring safety from BSE/TSE concerns. The final vaccine formulation does not contain any new or known hazardous excipients. Process performance qualification results showed the consistent elimination of all process residuals and impurities throughout the drug substance manufacturing process. There are no antibiotics used in the manufacturing process of ERVEBO. The vaccine contains no adjuvant or preservatives.

The vaccine manufacturing process is robust, and the virus titers achieved are consistent. The sponsor performs in-process and release testing of the vaccine and its intermediates at different stages of manufacturing to ensure that the product meets specifications and manufacturing is consistent.

Release testing for final drug product (frozen product) includes: bacterial and fungal sterility, bacterial endotoxin content, potency (virus concentration), virus identification, physical appearance, (b) (4), extractable volume, (b) (4), and total protein.

The acceptance specification for the potency of the vaccine is (b) (4). The minimum release specification of (b) (4) is based on the assessed stability profile. The expiry specification of 7.2×10^7 pfu/mL at the end of expiry period of 36 months was defined based on data from the clinical studies showing that the vaccine is immunogenic and protective at a dose of 7.2×10^7 pfu/mL, and therefore, a minimum stability potency specification of 7.2×10^7 pfu/mL is acceptable.

Based on the information submitted in the BLA, I recommend approval of the product.

B. RECOMMENDATION

I. APPROVAL

a. List of Drug Substance (DS) and Drug Product (DP) manufacturing facilities:

- Manufacture of DS: MSD (b) (4)
- Manufacture of DP: MSD (b) (4)

b. List of approvable Comparability Protocols:

- Not applicable

c. Post-Marketing Commitments Not Subject to The Reporting Requirements Under Section 506B

1. Provide the Final Drug Product process performance qualification final validation report.

d. List of Post-Marketing Agreements (PMAs):

The following CMC PMAs will be addressed by the applicant as described below and will not be included in the approval letter. These CMC PMAs below were submitted to the applicant on December 16, 2019.

1. To ensure process consistency, as part of your future continued process verification, please assess and, where appropriate, adjust process operating ranges. These ranges should reflect the actual manufacturing process. In addition, please address all discrepancies between target and operating ranges described in the manufacturing sections of the BLA and the blank batch records for the Drug Substance submitted to the BLA. Please submit the updated operating targets and ranges in the manufacturing sections of the BLA and the blank batch records covering all Drug Substance and Drug Product unit operations as a Prior Approval Supplement (PAS).
2. Provide data to support the requested total processing time of (b) (4) for the final Drug Product process, including the determination for a cumulative (b) (4). Submit the data as a Product Correspondence – Final Study Report.
3. Provide the final stability results for the ongoing studies of the Drug Substance and Drug Product PPQ lots when the stability studies are completed. Submit your final report as a Product Correspondence – Final Study Report.
4. Provide the final validation report for the Total Protein Test performed on the (b) (4) Drug Product to support the intended use of the assay and, specifically, to show that the assay results are not affected by the (b) (4) Drug Product matrix by assessing the spike recovery of the standard ((b) (4)) over the assay range and dilutional parallelism between the standard ((b) (4)) and the product over the assay range. Submit your final report as a Product Correspondence – Final Study Report.
5. Execute a one-time supplemental verification for the (b) (4) test, using a (b) (4) to confirm that the combination of medium used in the method and V920 matrix is (b) (4). Submit your final report as a Product Correspondence – Final Study Report.

6. Perform host cell protein (HCP) testing on future (b) (4) lots with a validated assay to characterize the level of residual HCP. Submit your final report and request to remove testing of these residuals as a Prior Approval Supplement (PAS) – Final Study Report.
7. A substantial portion of the CMC information that was submitted to the BLA in response to information requests in Module 1 was not included in the CMC sections in Module 3. Therefore, please include all updated CMC information such as description of in-process controls, test methods, validation reports, certificates of analysis, storage conditions, description of manufacturing steps, impurities, and deviation reports in the appropriate sections of Module 3. In Module 1 of your submission, please include a Table listing the CTD location of each update and a brief description of the revision. In addition, please identify and describe any specific information which is not identical to the information provided in the BLA prior to its approval. Submit the Module 3 updates as a Product Correspondence – Module 3 Final CMC Information Updates

e. Considerations for Inspectional Follow-up (e.g., flagging inspectional issues for future surveillance inspections)

- Review of executed batch records for the completed DS and DP lots.
- Review of stability results for the completed (b) (4) DP lots.
- Review of storage conditions and available number of (b) (4) vials.
- Review of information on source, specification, and qualification characteristics of the currently used lot of (b) (4) used in the identity test and the potency test, and information on lot-to-lot qualification of this reagent, if applicable.
- Review of test results for the absence of (b) (4) in the (b) (4).
- Review of test result for the (b) (4) test using a (b) (4) (such as (b) (4)) to confirm that the combination of medium used in the method and V920 matrix is (b) (4).

f. Considerations for Clinical Follow-up (e.g., flagging issues for future surveillance)

- If testing for vaccine virus viremia and shedding will be assessed in future clinical studies of ERVEBO, the RT-PCR method and validation information for the assay(s) should be submitted to the IND prior to their use.

g. Lot release requirements

The lot release protocol (LRP) is provided (amendments 4, 22, and 29) and found to be acceptable.

II. COMPLETE RESPONSE (CR)

Not applicable.

III. SIGNATURE BLOCK

Reviewer/Title/Affiliation	Concurrence	Signature and Date
Primary Level Review including all CMC reviewers	Concur	
Secondary Level Review (e.g., Branch/Lab Chief)	Concur	
Tertiary Level Review (e.g., Division Director)	Concur	

Review of CTD

Module 3

3.2.S DRUG SUBSTANCE

(b) (4)



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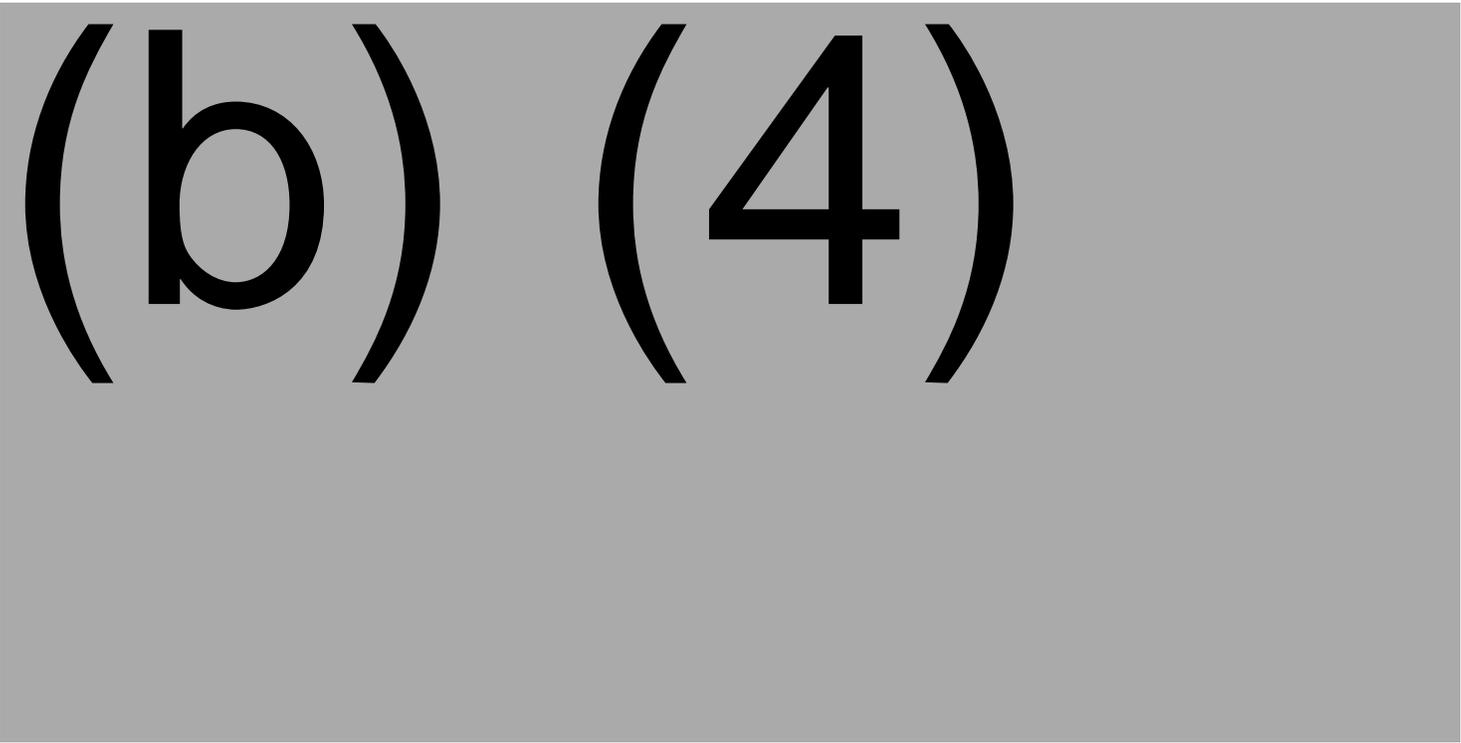


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(b) (4)



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(b) (4)

3.2.P DRUG PRODUCT

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

The vaccine Drug Product (DP) is a solution for injection manufactured by aseptic addition of the Bulk Drug Substance (BDS) to the Drug Product Stabilizer Solution, which consists of 2.5 g/L recombinant human serum albumin, 10 mM Tris, (b) (4), and Water for Injection. The vaccine is filled into single-dose vials to ensure recoverable volume of 1.0 mL for injection. The glass vial is stoppered and capped with aluminum overseals. The DP appears as a colorless to slightly brownish-yellow liquid with no particulates visible. No reconstitution diluent is used for this vaccine. The composition of the final DP per 1 mL dose is provided in the table below.

Table 56: Composition of the Drug Product

Active Ingredients	Concentration	Amount per 1 mL	Function
Recombinant Zaire ebolavirus Kikwit-95 envelope glycoprotein modified vesicular stomatitis virus	$\geq 7.2 \times 10^7$ pfu/mL	$\geq 7.2 \times 10^7$ pfu	Active immunogen
Inactive Ingredients	Concentration	Amount per 1 mL	Function
Tromethamine (Tris)	10 mM	0.01 mmol	Buffer
Recombinant Human Serum Albumin (rHSA)	2.5 g/L	2.5 mg	Stabilizer
Water for Injection	Not Applicable	Quantity Sufficient	Solvent

pfu: Plaque forming units. (b) (4) solution is used to adjust the (b) (4), if needed.

3.2.P.2 Pharmaceutical Development

3.2.P.2.1 Components of the Drug Product

3.2.P.2.1.1 Drug Substance

3.2.P.2.1.2 Excipients

(b) (4)

The Drug Product (DP) is a solution for injection made by (b) (4) BDS in the DP Stabilizer Solution, which consists of 10 mM Tris, 2.5 g/L rHSA, (b) (4), and Water for Injection. Two excipients are presented in the final Drug Product (Recombinant Human Serum Albumin and Tromethamine (Tris)). No excipients of animal or human origin are used. Recombinant Human Serum Albumin is used as a stabilizer in the DP. Recombinant Human Serum Albumin

is a common excipient used in the manufacture of vaccines and biologics. Tromethamine (Tris) is a buffer that maintains the vaccine pH within the optimal range for vaccine virus stability during processing and use. The target (b) (4) of the vaccine is (b) (4). Water for Injection is used as the solvent in which the Tromethamine and recombinant human serum albumin are solubilized.

3.2.P.2.2 Drug Product

3.2.P.2.2.1 Formulation Development

The Ebola Zaire Vaccine (rVSVΔG-ZEBOV-GP, live attenuated) DP is formulated in 10 mM Tris, (b) (4) with 2.5 mg/mL recombinant human serum albumin. Due to the urgent unmet medical need, the current Drug Product formulation has the same composition and pharmaceutical form as that used in historical clinical trials where safety and efficacy was established.

3.2.P.2.2.2 Overages

There are no overages for this product. An overfill, the volume filled in each container in slight excess of the labeled content, is provided to ensure that the minimum recoverable volume release criterion is met.

3.2.P.2.2.3 Physicochemical and Biological Properties

Potency, (b) (4) are tested as part of Drug Product release specifications. These tests and acceptance criteria are described in section 3.2.P.5. The other physical characteristics measured to characterize the Drug Product were (b) (4). For (b) (4), measurements were made on active Drug Product. For (b) (4), measurements were made on (b) (4). The reported values are provided in section 3.2.P.2.2. These characteristics are provided for general information only. They are not specifications and are not regularly measured. The density value is utilized to convert volume to weight for the drug product formulation and filling processes.

3.2.P.2.3 Manufacturing Process Development

The Ebola Zaire Vaccine DP manufacturing process consists of (b) (4) of BDS, (b) (4) of DP Stabilizer Solution (DPSS), formulation, filling (including stoppering), capping, inspection, labeling, secondary packaging, shelf freezing, DP storage, and DP shipping. The DP manufacturing process has been developed to ensure manufacturing robustness. The DP has been manufactured at the following facilities:

- (b) (4)
- Merck (b) (4)
- MSD (b) (4)

The tables below provide a detailed manufacturing history of the clinical, stability, emergency-use, and engineering batches of DP. Release testing results for batches used in clinical studies are provided in Section 3.2.P.5.4 Batch Analysis. Some of the batches made at Merck (b) (4) were in a (b) (4) image, in addition to the single-dose image. The commercial product will be available only in the single-dose image.

Table 57: History of Ebola Zaire Vaccine Drug Product Lots at (b) (4)

DP Batch Number	DP Date of Manufacture	DP Batch Size, (b) (4)	BDS Lot(s) Used	BDS (b) (4)	(b) (4)	Image	Material Disposition
(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	Single-Dose	Clinical Trials Stability Studies at -80°C
(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	Single-Dose	Clinical Trials Stability Studies at -80°C
(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	Single-Dose	Stability Studies at -80°C
(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	Single-Dose	Stability Studies at -80°C

(b) (4)	Single-Dose	Stability Studies at -80°C
	Single-Dose	Stability Studies at -80°C
	Single-Dose	Stability Studies at -80°C
	Single-Dose	Stability Studies at -80°C

*(b) (4)

Table 58: History of Ebola Zaire Vaccine Drug Product Lots at Merck (b) (4)

DP Batch Number	DP Date of Manufacture	DP Batch Size, (b) (4)	BDS Lot(s) Used	BDS (b) (4) (b) (4)	Image	Material Disposition
(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	Clinical Trials Stability Studies at -70°C
						Clinical Trials Stability Studies at -70°C
						Clinical Trials Stability Studies at -70°C
						Stability Studies at -80°C
						Emergency Use, Stability Studies at -70°C, (b) (4)
						Emergency Use, Stability Studies at -70°C, (b) (4)
						Emergency Use, Stability Studies at -70°C, (b) (4)
						Emergency Use
						Emergency Use
						Emergency Use

(b) (4)

Table 59: History of Ebola Zaire Vaccine Drug Product Lots at MSD (b) (4)

DP Batch Number	DP Date of Manufacture	Batch Size, (b) (4)	BDS Lot(s) Used	BDS (b) (4) (b) (4)	Image	Material Disposition
(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	Single-Dose	(b) (4) batch

Table below provides a high-level comparison of the Ebola Zaire Vaccine DP manufacturing process at the different sites.

Table 60: Comparison of Ebola Zaire Vaccine Drug Product Manufacturing Processes

Parameter	Drug Product Facility			Rationale for Change
	(b) (4)	Merck (b) (4)	MSD (b) (4)	
Purpose	Clinical Trials, Stability Studies	Clinical Trials, Stability Studies, Emergency-use	Engineering, Process Validation, Commercial	Transfer to commercial scale
Dosage Form	Frozen Liquid Vial	Frozen Liquid Vial	Frozen Liquid Vial	No change
Vial	Single-Dose (b) (4) tubing glass vial	Single-Dose 2 mL (b) (4) tubing glass vial	Single-Dose (b) (4) tubing glass vial, washed	The commercial product will only be provided in the single-dose image. All sites used (b) (4) tubing glass vials. The single-dose vials at (b) (4) have the same

		(b) (4)		dimensions as the (b) (4) vial with the exception of the minimum bottom wall thickness (0.70 mm for (b) (4) vs. 0.60 mm for (b) (4)). The vials for the MSD (b) (4) site come washed.
Stopper	Single-Dose 13 mm bromobutyl stopper	Single-Dose 13 mm chlorobutyl stopper, (b) (4) with (b) (4), ready for sterilization (b) (4)	Single-Dose 13 mm chlorobutyl stopper, (b) (4) with (b) (4) ready to use	The commercial product will use the same stopper as the single-dose image at Merck (b) (4). The stoppers for the MSD (b) (4) site come (b) (4) and do not require sterilization on-site.
Cap/Seal	Single-Dose 13 mm aluminum seal, with yellow or white flip-off cap	Single-Dose 13 mm aluminum seal, with red flip-off cap (b) (4)	Single-Dose 13 mm aluminum seal, with dark red plastic flip-off cap, (b) (4)	Changed color for commercial product which will only be provided in the single-dose image. The caps for the MSD (b) (4) site come sterilized via (b) (4).
Drug Substance Source	(b) (4)	(b) (4), Merck (b) (4)	MSD (b) (4)	Changed source to commercial manufacturing site.
Drug Product Stabilizer Solution	2.5 mg/mL Recombinant Human Serum Albumin and 10 mM Tris, (b) (4)	2.5 mg/mL Recombinant Human Serum Albumin and 10 mM Tris, (b) (4)	2.5 mg/mL Recombinant Human Serum Albumin and 10 mM Tris, (b) (4)	No change
Approximate Batch Size Range	(b) (4)	(b) (4)	(b) (4)	The commercial batch size is based on the commercial formulation vessel.
(b) (4)				
(b) (4) for DP Stabilizer Solution	Not (b) (4) during formulation process (Supplied as sterile)	(b) (4)	(b) (4)	The commercial process at MSD (b) (4) will use the same (b) (4) as the one used at Merck (b) (4).
Formulation Vessel	(b) (4)	(b) (4)	(b) (4)	The commercial formulation vessel at MSD (b) (4) is the same as the largest size used at Merck (b) (4).
Target Fill Weight (Range)	Single Dose (b) (4)	Single Dose (b) (4)	Single Dose (b) (4)	Commercial target and range based on achieving label claim of 1.0 mL per dose after accounting for variability of the commercial filling line and withdrawal losses.
Freezing Process and Final Drug Product Storage	Boxes of DP vials placed in freezer. Frozen DP stored at ≤ -60°C	Boxes of DP vials placed in freezer. Frozen DP stored at -70°C	Cartons of DP vials in cases placed in freezer. Frozen DP stored at -80°C to -60°C	DP at the MSD (b) (4) site is labeled and packaged with the commercial secondary packaging components prior to freezing.

*(b) (4) process introduced at (b) (4) starting with batch (b) (4).

A risk assessment approach was used throughout the development of the Ebola Zaire Vaccine DP manufacturing process. Process risk assessments were conducted during process development as well as prior to Process

Performance Qualification (PPQ). Development studies were identified and executed based on the initial risk assessment. The executed studies are summarized in the table below. A combination of studies across development and commercial scales was used to support the final commercial manufacturing process.

Table 61: Drug Product Manufacturing Process Development Overview

Process Step	Development Studies
(b) (4) Development	Effect of (b) (4) on Potency (Section 3.2.1) Determination of (b) (4) on Commercial Equipment (Section 3.2.2)
Drug Product Stabilizer Solution (b) (4) Development	Lab Scale (b) (4) Study (Section 3.3.1) Commercial Scale (b) (4) Study (Section 3.3.2) (b) (4) Studies (Section 3.3.3) Maximum (b) (4) Time (Section 3.3.4) (b) (4) Test Criteria (Section 3.3.5)
Drug Product Formulation Targeting	Drug Product Formulation Targeting (Section 3.4)
Formulation Mixing Development	Determination of Mixing Parameters (Section 3.5.1) Engineering Batch Parameters and Results – Formulation Mixing (Section 3.5.2)
Volume of Fill Determination	Approach (Section 3.6.1) Minimum Fill Volume (Section 3.6.2) Fill Target (Section 3.6.3) Engineering Batch Parameters and Results – Volume of Fill (Section 3.6.4)
Filling Process Development	Filling Start-up Studies (Section 3.7.1) Steady State Dosing Runs (Section 3.7.2) Filling Downtime Studies (Section 3.7.3) End of Fill Study (Section 3.7.4) Engineering Batch Parameters and Results – Filling (Section 3.7.5)
(b) (4)	(b) (4) (Section 3.8)
Drug Product Freezing Development	Lab-Scale Freezing Rate Study (Section 3.9.1) Commercial Scale Freeze Down Process Development (Section 3.9.2) Engineering Batch Parameters and Results – Drug Product Freezing (Section 3.9.3)
(b) (4)	(b) (4) (Section 3.10)

A commercial scale engineering batch was conducted at the commercial manufacturing site from (b) (4). The engineering batch was used to evaluate the potency model as well as process parameter set-points and ranges for the formulation through freezing processes. Actual process parameters used for the engineering batch fall within the ranges anticipated for the commercial process. Release testing was executed on the batch with passing results for all tests. The engineering batch release results are presented in Section 3.2.P.5.4 Batch Analysis, were reviewed and found to be adequate. In summary, the testing results for the engineering batch were within the proposed specifications for commercial manufacturing. Therefore, the DP process at the (b) (4) facility is capable of successfully manufacturing DP using the proposed potency model. Results and conclusions from this engineering batch will be leveraged to finalize parameter set-points and ranges for Process Performance Qualification (PPQ) batches.

3.2.P.2.4 Container Closure System

The DP container closure system consists of the primary packaging components listed in the table below.

Table 62: Primary Packaging of Ebola Zaire Vaccine Drug Product

Component	Description
Vial	2.0 mL - (b) (4) borosilicate clear tubing glass vial, 13 mm finish
Vial Stopper	13 mm (b) (4) coated (b) (4)
Cap/Seal	13 mm, aluminum seal with dark red plastic flip-off cap

Note: The cap does not come in contact with the Drug Product.

The suitability of the container closure system for the DP is demonstrated by the following:

- Compendial testing and ISO standards of primary packaging components
- Assessment of extractables and leachables

Suitability of the container closure system is further demonstrated by the following studies presented in other sections, as indicated:

- Container Closure Integrity [Sec. 3.2.P.3.5.2]
- Stability [Sec. 3.2.P.8.1] and Stability Data [Sec. 3.2.P.8.3]
- (b) (4) [Sec. 3.2.P.8.1] and Stability Data [Sec. 3.2.P.8.3]
- Vial cartons are opaque and intended to protect from light [Sec. 3.2.P.7.1]

The (b) (4) Borosilicate glass vials and the (b) (4) stoppers have been evaluated for extractables and the results are discussed in section 3.2.P.2.4. The aluminum seal cap is not evaluated for extractables since it does not come into contact with product during product filling, storage or distribution. The assessment of extractables and leachables for this container closure system was performed and found to be acceptable (see section 3.2.P.2.4).

3.2.P.2.5 Microbiological Attributes

Ebola Zaire Vaccine is provided in single-dose vials with no preservative. The DP is manufactured by aseptic addition of sterile BDS and DP Stabilizer Solution. The formulated (b) (4) is aseptically filled into vials. Sterility testing is included as part of release testing for every lot. Process simulations verify the robustness of the aseptic processing steps. Additional integrity of the filled vials is provided by Container Closure Integrity (CCI) Validation [Section 3.2.P.3.5.2].

3.2.P.2.6 Compatibility

Compatibility of Ebola Zaire Vaccine DP with the selected vial and stopper container closure system (CCS) is demonstrated by development of the container closure system and Drug Product stability studies. The compatibility of Ebola Zaire Vaccine DP with the process contact materials (PCMs) during the manufacturing process was evaluated through a risk assessment, which was performed for all the PCMs to understand the risk of extractables and leachables under commercial manufacturing conditions. PCMs in the Ebola Zaire Vaccine DP manufacturing process were assessed using a risk-based approach for potential impact on product quality and patient safety, including risk of leachables. A list of the PCMs is provided. The PCMs in the process were confirmed to be appropriate for use and pose no risk of extractables and leachables.

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

The information provided is acceptable.

3.2.P.3 Manufacture

3.2.P.3.1 Manufacturer(s)

The sites responsible for manufacturing, testing, packaging, labeling, release, and storage of Ebola Zaire Vaccine Drug Product are presented in the table below.

Table 63: Manufacturing, Testing, Packaging and Release Sites for Ebola Zaire Vaccine Drug Product

Name and Address of Site	Responsibility
MSD (b) (4)	<ul style="list-style-type: none"> • Drug Product manufacturing • Drug Product Stabilizer Solution In-Process testing during formulation process • Drug Product inspection, labeling and secondary packaging • Drug Product storage (packaged vials) • Final Drug Product release onto the market
(b) (4)	<ul style="list-style-type: none"> • Drug Product release testing
(b) (4)	<ul style="list-style-type: none"> • Drug Product release testing
(b) (4)	<ul style="list-style-type: none"> • Drug Product release testing and stability testing
(b) (4)	<ul style="list-style-type: none"> • Drug Product stability testing

3.2.P.3.2 Batch Formula

Ebola Zaire Vaccine DP is prepared by combining BDS with Drug Product Stabilizer Solution. Formulation batches range from (b) (4). The amounts of BDS and Drug Product Stabilizer Solution in a formulation batch depend on the BDS (b) (4) and volume required to ensure that the DP potency is within specification at the time of release. The composition of the DP is provided above. The Drug Product Stabilizer Solution and (b) (4) both contain the (b) (4) Tris and rHSA. Regardless of amounts of BDS and Drug Product Stabilizer Solution added, the Tris and rHSA concentrations remain constant. (b) (4) PPQ lots were manufactured to initially validate the commercial process. Batch formulas for only one of these PPQ lots is provided in the table below. The PPQ batch formula data, generated in line with the PPQ Protocol provided in section 3.2.R Regional Information, will be provided post-licensure.

Table 64: Batch Formulas for Process Performance Qualification Lots

Component	Formulation Batch Number
Batch Size (b) (4)	(b) (4)
Total Bulk Drug Substance (BDS) (b) (4)	
Drug Product Stabilizer Solution (b) (4)	

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

One deficiency was identified in this section. It was addressed and resolved successfully through a request for additional information from the sponsor. The response provided by the sponsor in amendment 35 is considered acceptable (see below).

Agency Question:

Please provide information on batch numbering and pooling scheme for the formulation of final drug product.

Company Response:

The batch number consists of ten digits which reflect 8 entities of information. The batch number is built as follows: H-JJ-W-NN-X-M-Y-Z

Where:

(b) (4)

The drug product formulation calculations are performed in a validated spreadsheet described in SOP HH-DP/0033E included in this response.

3.2.P.3.3 Description of Manufacturing Process

Ebola Zaire Vaccine DP is manufactured as a sterile, aseptically filled solution into a single-dose ISO standard 2R vial. The DP batch size range is (b) (4) for the (b) (4). Each (b) (4) vial is filled to achieve a label claim of 1.0 mL. The formulation process consists of (b) (4)

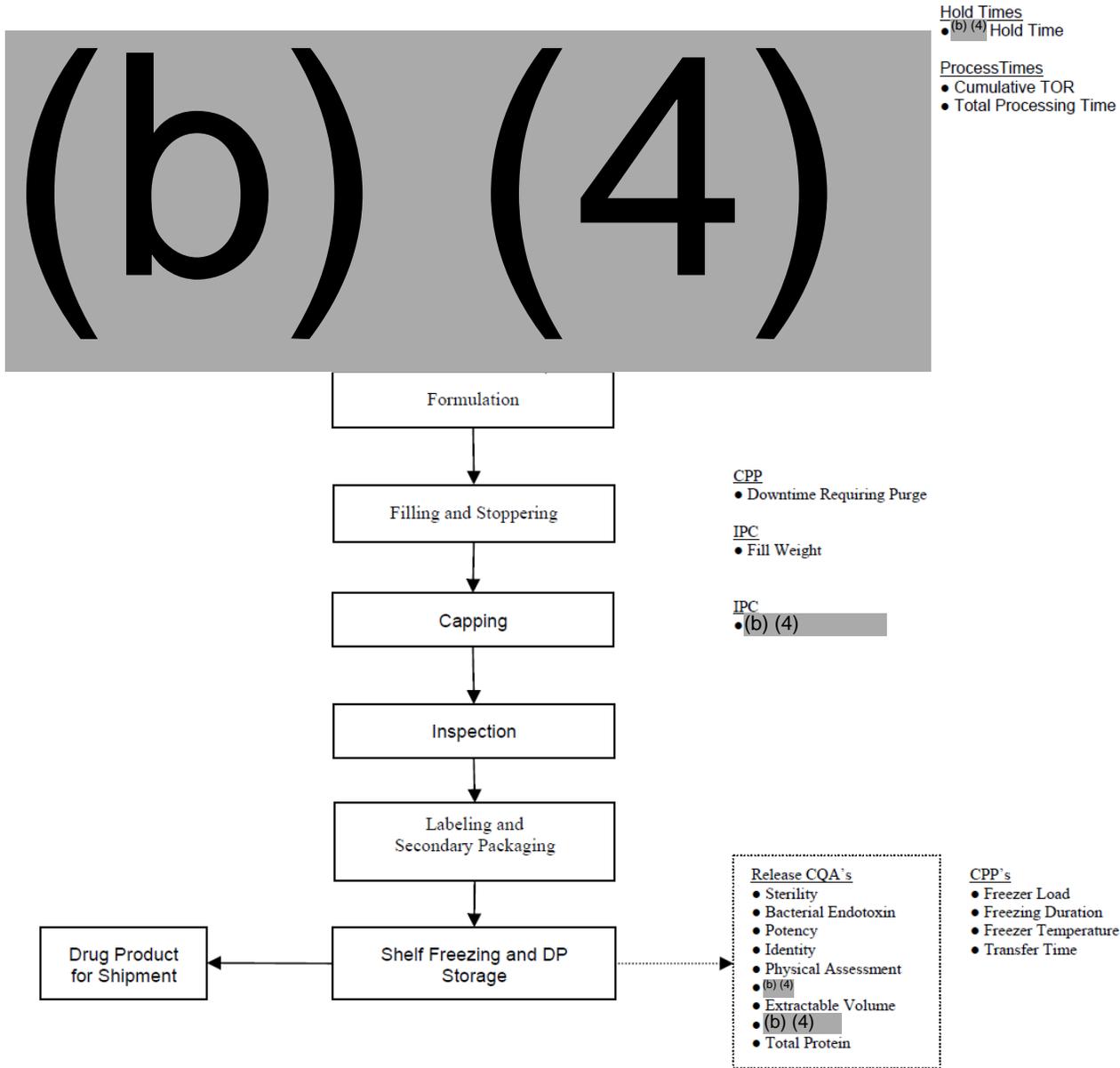
The aseptic formulation process is performed under Grade (b) (4) environmental conditions using (b) (4) processing.

The (b) (4) is subsequently filled, aseptically, into vials using (b) (4) and the vials are then stoppered. The filling process takes place in a Grade (b) (4) Restricted Access Barrier System (RABS). Stoppered vials are transferred into the capping room using (b) (4) that provide Grade (b) (4) air supply and sealed under Grade (b) (4) air supply. The filled and sealed vials are then inspected, labeled, and packaged prior to freezing and storage at -80°C to -60°C. Appropriate measures are taken throughout the manufacturing process to limit the time the product is (b) (4). The temperature range

is controlled by means of temperature control and temperature alarms. Standard environmental and in-process controls minimize exposure to light during the manufacturing process.

A process flow diagram of the formulation, filling, capping, inspection, packaging, and freezing processes is shown in Figure 4 below (also see BLA section 3.2.P.3.3, figure 1). Critical Process Parameters (CPPs), in-process samples, and release sample points are indicated in the diagram.

Figure 4: Process Flow Diagram for the Formulation, Filling, Capping, Inspection, and Packaging Processes



The (b) (4) is stored in (b) (4), filled with up to (b) (4). Each (b) (4) is stored in a storage container and is stored (b) (4) until needed in the DP manufacturing process.

Drug Product Stabilizer Solution

The DP stabilizer solution is manufactured at (b) (4). Upon receipt at the DP stabilizer solution manufacturer (i.e., (b) (4)), the rHSA is at minimum tested for (b) (4) prior to use. Formulation of the stabilizer solution entails (b) (4)

(b) (4). The DP Stabilizer Solution is then delivered to the Merck (b) (4). The release criteria for the DP Stabilizer Solution are provided in section 3.2.P.4.1. The DP Stabilizer Solution is stored at (b) (4) before transfer to the Grade (b) (4) formulation suite. The container of the DP Stabilizer Solution is (b) (4) formulation vessel by aseptic connections. The DP Stabilizer Solution is passed through (b) (4) via a (b) (4). An initial (b) (4) is discarded as a (b) (4), and a (b) (4) sample is taken, with a limit of (b) (4). The (b) (4) is confirmed (b) (4).

(b) (4) Drug Product Formulation Process

(b) (4)

(b) (4) The start of the processing time is when (b) (4). Studies performed to establish (b) (4) conditions are described in section 3.2.P.2.3.1 Manufacturing Process Development.

Prior to the start of manufacture, the required amounts of DP Stabilizer Solution and BDS are calculated based on (b) (4) the target potency of the (b) (4) to ensure that the DP potency and (b) (4) will be within specification at release. Formulation is performed in a Grade (b) (4) room using (b) (4) processing. The (b) (4) batch size range is (b) (4), with a target range of (b) (4) based on process development studies. The formulation process consists of (b) (4)

(b) (4)

(b) (4) Once dose is set, the vials are filled using (b) (4) with a fill weight of (b) (4). Excess volume is included during filling to ensure recovery of the label claim of 1.0 mL per vial of DP. Studies performed to establish the required overfill are described in section 3.2.P.2.3.1 Manufacturing Process Development. Dose is controlled by (b) (4). The (b) (4) are adjusted, if necessary, to maintain dose within a specified range around the target. The filling line is purged for a minimum of (b) (4) vials if the allowable downtime limit of (b) (4) is exceeded during the fill, or a minimum of (b) (4) vials are purged if a downtime exceeds (b) (4). A contingency process has been established to aseptically disconnect and reconnect the (b) (4) to minimize exposure to (b) (4)

(b) (4) in the event of an extended downtime. (b) (4)

The vials are stoppered with 13 mm chlorobutyl stoppers that are supplied Ready-to-Use (RTU) by the vendor. Filled and stoppered vials are automatically inspected for missing stoppers prior to being loaded onto stainless steel trays. The trays of filled vials are then transferred into (b) (4) that provide Grade (b) (4) air supply and then manually transported to the capping room. Capping occurs under Grade (b) (4) air supply in a RABS with a Grade (b) (4) background. Vials are loaded onto the capping machine and automatically inspected for raised or missing stoppers prior to applying the seal. Aluminum seals are applied and then crimped. Seals are supplied (b) (4) by the vendor and supplied Cleaned, Certified, Sterile (CCS). (b) (4) is tested at the beginning and end of the capping process for each capping head with a specification of (b) (4). Capped vials are transferred into trays with lids, placed on a cart, and stored at (b) (4) prior to inspection. Trays of vials are taken out of (b) (4) storage and then the vials are 100% manually inspected. After the inspection, the vials are returned to storage at (b) (4). In order to minimize RT exposure, a limited number of trays of vials are removed from storage for inspection at a given time.

Trays of inspected vials are taken out of (b) (4) storage and then the vials are labeled using an automatic labeling machine, which performs a 100% verification of labeling. The vials are manually packed into 10-vial cartons with package inserts and loaded into shipping cases. Up to (b) (4) cartons are placed into each shipping case. The cases of packaged vials can be stored at (b) (4) to freezing if not directly transferred to the (b) (4) freezers.

The cases of packaged vials are transferred to (b) (4) freezers dedicated to freezing the DP, with a limit of (b) (4) cases per freezer. The end of DP processing time is triggered when the last case of packaged vials is placed in the freezer. The cases of packaged vials are held in the (b) (4) freezer for a minimum of (b) (4) to complete the initial freezing of the DP to -80°C to -60°C. After the minimum initial freeze time, release samples are taken, and the cases of frozen packaged vials are transferred to long term storage at -80°C to -60 °C. When transferring cases of frozen packaged vials, there is a limit of (b) (4) until either the cases of frozen vials are in the storage freezer or in a cooler/container with dry ice.

The DP is temperature-sensitive. Stability studies have been performed to quantify the kinetics of potency loss at both room temperature (RT) and 2°C to 8°C. The current out of refrigeration and exposed to RT conditions (Time Out of Refrigeration, TOR) have been determined. (b) (4)

(b) (4). In addition, the current manufacturing process controls allow for (b) (4) of total process time (including up to (b) (4)) from the time the (b) (4) to the time when the last case of packaged DP is placed in the (b) (4).

(b) (4)

The planned manufacturing controls to limit exposure to RT conditions are also sufficient to (b) (4). Final container

vials are shipped in thermal protective systems qualified to maintain an internal temperature of <-60°C for the duration of shipment. The DP is protected from light during shipping by means of the secondary packaging.

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

Several deficiencies was identified in this section. They were addressed and resolved successfully through a request for additional information from the sponsor. The responses provided by the sponsor in amendment 51 are considered acceptable (see below).

Agency Question:

Regarding the manufacturing process and controls for the formulation step:

- a. Please provide the target (b) (4) applied to the formulation step in sections 3.2.P.3.2 and 3.2.P.3.3.
- b. Please provide information on the operating ranges for (b) (4), including the (b) (4) (e.g., (b) (4) duration and (b) (4). Please include this information in sections 3.2.P.3.3 and supportive data under process validation (section 3.2.P.3.5), as applicable.

Company Response:

- a. To avoid redundancy and simplify future lifecycle management, we propose to only add the target (b) (4) to section 3.2.P.3.3 (included herein).
- b. The operating ranges for (b) (4) formulation, including the (b) (4) duration and (b) (4) were added to section 3.2.P.3.3. Merck will add the values from PPQ to section 3.2.P.3.5.1 when data are added for DP PPQ lots (b) (4). Please also see the development data located in section 3.2.P.2.3.1. Please see follow-up recommendations in section 9.B.I.d List of Post Marketing Agreements (PMAs).

3.2.P.3.4 Controls of Critical Steps and Intermediates

The process parameters for the manufacturing process of Ebola Zaire Vaccine DP were selected based on laboratory-scale experiments, full-scale developmental studies, and a process risk assessment (RA), described in Section 3.2.P.2.3.1 Manufacturing Process Development. As part of the control strategy, critical process parameters (CPPs) were defined.

The control strategy for Ebola Zaire Vaccine DP is comprised of process inputs and outputs including critical Process Parameters (CPPs), in-process controls (IPCs), and critical quality attributes (CQAs). CPPs and IPCs were determined using a decision tree analysis based on potential and extent of impact to CQAs. The tables below summarize the CPPs, IPCs, acceptance criteria, and impacted CQAs, as well as the critical hold times and process times.

Table 65: Critical Process Parameters for Ebola Zaire Vaccine Drug Product

Process Step	Process Parameter	Acceptance Criteria	Classification	Impacted CQA(s)
(b) (4)	(4)		CPP	(b) (4)
			CPP	
			IPC	
			IPC	

Filling	Downtime Requiring (b) (4)	(b) (4)	CPP	Extractable Volume
Filling	Fill Weight	(b) (4)	IPC	Extractable Volume
Capping	(b) (4)	(b) (4)	IPC	Sterility
Shelf Freezing	Freezer Load	(b) (4)	CPP	Potency
Shelf Freezing	Freezing Duration	(b) (4)	CPP	Potency
DP Storage	Transfer Time	(b) (4) until either the cases of frozen vials are in the storage freezer (b) (4)	CPP	Potency
DP Storage	Freezer Temperature	-80°C to -60°C	CPP	Potency

Table 66: Hold Times and Process Times for the DP

Condition	Classification	Operating Range
(b) (4) Hold Time at (b) (4)	Hold Time	(b) (4)
Cumulative Time (b) (4)	Process Time	(b) (4)
Total Processing Time	Process Time	(b) (4)

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

The information currently provided for DP Controls of Critical Steps and Intermediates is acceptable. Results of Process Validation is available for only one PPQ DP batch (b) (4) at this time. Final PPQ DP Process Validation report will be submitted post approval. Please see follow-up recommendation in section 9.B.I.c List of Post-Marketing Commitments (PMCs)/Post-Marketing Requirements (PMRs) and recommendation regarding hold times applied for DP filling steps in section 9.B.I.d List of Post Marketing Agreements (PMAs).

3.2.P.3.5 Process Validation and/or Evaluation

The consistency of DP manufacturing during formulation, filling, capping, inspection, labeling, packaging, and freezing are confirmed through an analysis of the PPQ batches. (b) (4)

The formulation batches and fill and package batches that were manufactured in support of PPQ are listed in the tables below. A recovery process has been established to (b) (4)

. This recovery process will be

(b) (4)

(b) (4)

(b) (4)

Process Performance Qualification Only Tests

There are several tests which were only performed for the PPQ lots and are presented in the table below. These tests and specifications will not be applied to routine testing.

Table 71: Proposed PPQ Only Tests

Test	Specification
Residual Benzonase ^a	≤ 15 ng/dose
Residual Host Cell DNA ^a	≤ 10 ng/dose
General Safety Testing	No death or weight loss

^aTest is performed on (b) (4), but the specification is applied to the Drug Product by calculation.

Residual Benzonase and Residual Host Cell DNA Test

This test quantifies the amount of residual Benzonase and the amount of residual host cell DNA in the (b) (4) and is described in section 3.2.S.2.5.3. The residual Benzonase and residual host cell DNA of the (b) (4) is used to calculate the amount of residual Benzonase and residual host cell DNA present in the Ebola Zaire Vaccine DP based on the (b) (4) during formulation and filling. Reference to this test is included

in this DP section since a specification exists for the Process Performance Qualification lots for residual Benzonase in Ebola Zaire Vaccine DP, even though the material is not directly tested.

General Safety Test Procedure

The General Safety test is an *in vivo* purity test for extraneous agents. This test was performed on the PPQ lots only. This test has not been validated, since typical validation parameters cannot be applied meaningfully to *in vivo* tests.

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

The information provided is acceptable. Results of Process Validation is available for only one PPQ DP batch (b) (4) at this time. Final PPQ DP Process Validation report will be submitted post approval.

3.2.P.4 Control of Excipients

3.2.P.4.1 Specifications

The excipient used in Ebola Zaire Vaccine is a preformulated purchased Drug Product Stabilizer Solution. The qualified supplier formulates the DP stabilizer by solubilizing 10mM Tromethamine (Tris), (b) (4), and 2.5g/L recombinant human serum albumin (rHSA) in Water for Injection, (b) (4). Upon receipt, the pre-formulated DP Stabilizer Solution is tested to the specifications listed in the table below.

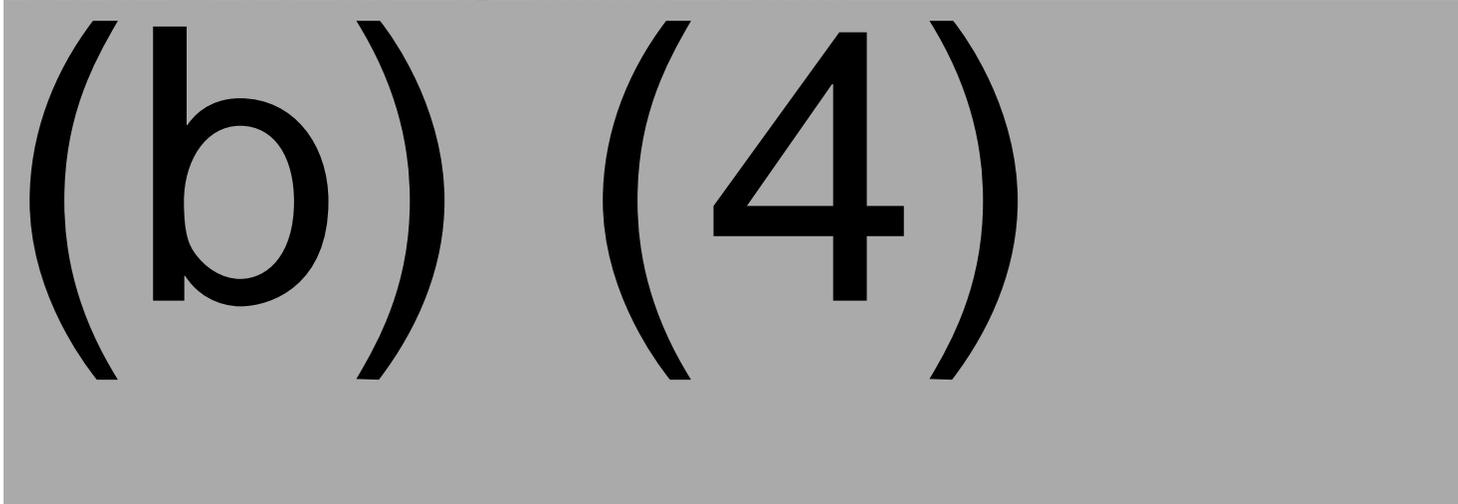
The rHSA is a novel excipient and further details are provided in section 3.2.A.3.

Table 72: Excipient in Ebola Zaire Vaccine

Excipient	Ingredient	Ingredient Concentration	Amount per 1 mL
Drug Product Stabilizer Solution	Tromethamine (Tris), (b) (4)	10 mM	0.01 mmol
	Recombinant Human Serum Albumin (rHSA)	2.5 g/L	2.5 mg
	Water for Injection, (b) (4)	Not Applicable	Quantity Sufficient

(b) (4) solution is used to adjust (b) (4), if needed.

Table 73: Tests Performed on Drug Product Stabilizer Solution



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

The analytical procedures used for testing the excipients, for (b) (4) are performed in compliance with the relevant (b) (4). The validation status of tests for the Drug Product Stabilizer Solution is provided in the table below.

Table 74: Validation Status for Drug Product Stabilizer Solution Tests



3.2.P.4.4 Justification of Specifications

Specifications and justifications for tests performed for the excipient DP Stabilizer Solution are also provided in section 3.2.P.4.4. The information is reviewed and found to be adequate.

3.2.P.4.5 Excipients of Human or Animal Origin

No human- or animal-derived materials are used in the manufacture of the DP excipient.

3.2.P.4.6 Novel Excipient

Rice-derived recombinant Human Serum Albumin (rd-rHSA) is used as a stabilizer component in the Ebola Zaire Vaccine. The rd-rHSA is a novel excipient, as such details of manufacture, characterization and controls for the rd-rHSA is provided in section 3.2.A.3 Novel Excipients.

Overall Reviewer’s Assessment of Section 3.2.P.4:
The information provided is acceptable.

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 release tests, specifications and justification of the acceptance criteria for the assays used for release and stability testing of Ebola Zaire Vaccine Drug Product are shown in the tables below. This testing confirms the absence of extraneous agents, verifies potency and identity, and provides a measure of quality and process consistency.

Table 75: Release Test Methods and Specifications – Drug Product

Test	Acceptance Criteria	Justification of Acceptance Criteria	
Sterility	No growth	Detectable growth indicates bacterial or fungal contamination.	
Bacterial Endotoxins	(b) (4)	Consistent with the recommendation from (b) (4) and based on the (b) (4) requirement of (b) (4), the specification is calculated as such: (b) (4)	
Potency	(b) (4)	See Section 1.1	
Identity	Confirm rVSVΔG-ZEBOV-GP	(b) (4) will confirm identity of Ebola Zaire Vaccine.	
Physical Assessment	Color	Expected physical appearance of the Ebola Zaire Vaccine Drug Product (rVSVΔG-ZEBOV-GP, live attenuated)	
	Opalescence		No visible particulates
	Particulates		
(b) (4)	(b) (4)	(b) (4)	

		(b) (4)
Extractable Volume	The (b) (4) volume of (b) (4) containers is no less than (b) (4)	To demonstrate that the volume on the label can be consistently extracted from the Drug Product containers.
(b) (4)	(b) (4)	Based on process capability and consistent with the (b) (4)
Total Protein	(b) (4)	Ensures the final formulation step, addition of rHSA at a target concentration of 2.5 mg/mL, is performed correctly.

Table 76: Stability Test Methods and Specifications – Drug Product

Test	Acceptance Criteria	Justification of Acceptance Criteria	
Sterility	No growth	Detectable growth indicates bacterial or fungal contamination.	
Potency	$\geq 7.2 \times 10^7$ pfu/mL	See Section 1.1	
Physical Assessment	Color	Expected physical appearance of the Ebola Zaire Vaccine Drug Product (rVSVΔG-ZEBOV-GP, live attenuated)	
	Opalescence		No visible
	Particulates		particulates
(b) (4)	(b) (4)	(b) (4)	
Container Closure Integrity	Pass	Ensures integrity of the container closure system through shelf life of the product	

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

One deficiency was identified in this section. It was addressed and resolved successfully through a request for additional information from the sponsor. The response provided by the sponsor in amendment 52 is considered acceptable (see below).

Agency Question:

We note that the identity test is performed on the drug product (Module 3.2.P.5.1, Table 1). However, it is not clear if this test is performed after all labeling operations are concluded, as required by 21 CFR 610.14. Please clarify. If the identity test is not performed on the drug product after all labeling operations are concluded, please institute this test on labeled final container product.

Company Response:

The PPQ identity samples were taken prior to labeling operations. The sample plan will be updated so that the identity samples will be taken after all labeling operations are concluded starting on the next DP batch (DP (b) (4) – the first Commercial Batch).

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

The release test methods that are common to the (b) (4) DP (potency, identity, physical assessment (b) (4)) are described in section 3.2.S.4.2. The tests specific to the DP (endotoxin, extractables, (b) (4) and total protein) are described below.

Bacterial endotoxins

The (b) (4) method is used to quantify the (b) (4) bacterial endotoxin level in the Ebola Zaire Vaccine Drug Product. The test is performed in accordance with (b) (4)

Extractable volume

The purpose of this procedure is to measure the extractable volume of Ebola Zaire Vaccine Drug Product. Briefly, (b) (4)

The test is performed in accordance with (b) (4)

(b) (4)

Total protein

The purpose of this procedure is to determine the total protein concentration of Ebola Zaire Vaccine Drug Product using the (b) (4) Protein Assay. Briefly, (b) (4)

This test and its validation/matrix qualification results were reviewed by an assay expert from DBSQC assigned to this BLA submission. The method was found to be acceptable for its intended use; however, the DBSQC reviewer has noted that the validation package for this test is incomplete, and CBER informed the sponsor to provide the final validation report for this total protein test post-licensure, specifically, to show that the assay results are not affected by the (b) (4) Drug Product matrix by assessing the spike recovery of the standard ((b) (4)) over the assay range and (b) (4) between the standard ((b) (4)) and the product over the assay range. Please see follow-up recommendations in introduction item 9.B.I.d List of Post Marketing Agreements (PMAs).

Container closure integrity test

The integrity of the Ebola Zaire Vaccine Drug Product container closure system is assessed by utilizing a (b) (4) method. This test and its validation results were reviewed by a test expert from DMPQ assigned to this BLA submission. The method was found to be acceptable for its intended use.

Validation status for all the release and stability tests, as well as for the tests performed only on the PPQ lots is provided in the tables below. Compendial tests detailed in the (b) (4) were validated in compliance with the requirements of the (b) (4). All the acceptance criteria were satisfied.

Table 77: Release and Stability Testing – Drug Product

Test	Release/Stability Test	Testing Location	Method Number / Title	Validation Status
Sterility	Release and Stability	(b) (4)	1-P-QM-WI-9014676: Sterility Testing by (b) (4)	Compendial; verification complete
Bacterial Endotoxins	Release	(b) (4)	PROC-BREL_US-OP-035064(OPBT3002): Procedure for the (b) (4) Assay using the (b) (4) Method	Validated by vendor; V920 matrix qualified in validated method
Potency	Release and Stability	(b) (4)	1-P-QM-WI-9034077: Client Specific – V920 (b) (4) Assay, Merck & Co. Inc.	Validated
Identity	Release	(b) (4)	1-P-QM-WI-9044684: Client Specific, Confirmation of V920 Identity by (b) (4), Merck & Co. Inc.	Validated
Physical assessment (color, opalescence, particulates)	Release and Stability	(b) (4)	1-P-QM-FOR-9023679: Physical Assessment for Biological Products, Merck & Co. Inc. ((b) (4))	Compendial; verification complete
(b) (4)	Release and Stability	(b) (4)	(b) (4)	Compendial; verification complete
Extractable Volume	Release	(b) (4)	(b) (4)	Compendial; verification complete
(b) (4)	Release	(b) (4)	PROC_BREL-OPBT2167: (b) (4) Determination, GMP Using the (b) (4)	Validated by vendor; V920 matrix qualified in validated method
Total Protein	Release	(b) (4)	OPBT2148: Total Protein (b) (4), GMP	Validated by vendor; V920 matrix qualified in validated method
Container Closure Integrity	Stability	(b) (4)	TP-13104-00-012918-C101: (b) (4) Program: Sample Analysis Protocol, V920	Validated

Table 78: PPQ only and In-Process Tests

Test	Test Purpose	Testing Location	Method Number / Title	Validation Status
General Safety Testing	(b) (4) (Drug Product)	Merck Sharp & Dohme Corp. 770 (b) (4)	052650221GEN: General Safety Testing	Not validated since typical validation parameters cannot be applied meaningfully to in vivo tests.
Bioburden*	Drug Product (b) (4)	MSD (b) (4)	QC/1806: (b) (4)	Matrix suitability study is completed.

Note: The bioburden testing of the DP (b) (4) is not planned to be changed post-licensure.

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

The information provided is acceptable. Validation reports and detailed descriptions (or SOPs) of all non-compendial test methods are provided in the BLA, reviewed and found to be acceptable. The methods validations/qualifications were adequately performed to assure that these methods are suitable for their intended purpose.

3.2.P.5.4 Batch Analyses

The Drug Product lots listed below have been utilized in clinical studies as described in the table below.

Table 78: List of Drug Product Lots Used in Clinical Studies

Bulk Drug Substance Lot#	Drug Product Lot# (date of manufacture)	Clinical Study Utilization
(b) (4)	(b) (4) Lot: 003 05 13 (b) (4)	Protocol V920-001: WRAIR, USA Protocol V920-002: NIH/NIAID, USA Protocol V920-003: CCV, Halifax, Canada Protocol V920-004: NewLink, USA Protocol V920-005: WHO, Geneva, Switzerland Protocol V920-006: WHO, Hamburg, Germany Protocol V920-007: WHO, Gabon, Africa Protocol V920-008: WHO, Kenya, Africa Protocol V920-009: NIH/NIAID/PREVAIL, Liberia, Africa
(b) (4)	(b) (4) Lot: 001 10 14 (b) (4)	Protocol V920-009: NIH/NIAID/PREVAIL, Liberia, Africa Protocol V920-010: WHO, MSF, NIPH, Guinea, Africa Protocol V920-011: CDC, SLCMAHS, SLMHS/STRIVE, Sierra Leone, Africa Protocol V920-012: MSD/BARDA, USA, Canada, Europe
(b) (4)	Merck (b) (4) Lot: WL00060577 (b) (4) WL00060666 (b) (4)	Protocol V920-012: MSD/BARDA, USA, Canada, Europe
(b) (4)	Merck (b) (4) Lot: WL00060666 (b) (4)	Protocol V920-012: MSD/BARDA, USA, Canada, Europe
(b) (4)	Merck (b) (4) Lot: WL00061283 (b) (4)	Protocol V920-012: MSD/BARDA, USA, Canada, Europe

The release testing and results for the DP lots manufactured for clinical use at (b) (4) and Merck (b) (4) are provided below. The test methods and specifications listed below reflect the methods and specifications in place at the time of testing for clinical studies.

Table 79: Release Testing Results for Clinical Drug Product Lots Manufactured at (b) (4) (these lots were used in the clinical studies (safety & efficacy) to support the licensure)

Test	Specification	003 05 13	001 10 14
Sterility	No growth of bacteria and fungi detected	No growth of bacteria and fungi detected	No growth of bacteria and fungi detected
Bacterial Endotoxins	(b) (4)	(b) (4)	(b) (4)
Potency	(b) (4)	(b) (4)	(b) (4)
Identity	Confirm rVSVΔG-ZEBOV-GP	Presence of insert ZEBOV-GP	Identity of VSVΔG-ZEBOV-GP confirmed
Test for identity/Potency (b) (4)	Presence of Filovirus protein	Presence of insert Filovirus protein	Presence of Filovirus protein
Total DNA Content	Report result	(b) (4)	<5 ng/mL
Residual Benzoylase	Report result	1.326 ng/mL	0.48 ng/mL

Physical Appearance	Report results (for (b) (4)); Clear to slightly turbid and colorless solution (for (b) (4))	Clear, colorless solution	Clear, colorless solution
(b) (4)	Report result	(b) (4)	(b) (4)
Extractable Volume	(b) (4) 1.0 mL	(b) (4)	(b) (4)
(b) (4)	Report result	(b) (4)	(b) (4)
General Safety Testing (Mice)	No toxicity observed in mice	No toxicity observed in mice	No toxicity observed in mice
General Safety Testing (Guinea pigs)	No toxicity observed in guinea pigs	No toxicity observed in guinea pigs	No toxicity observed in guinea pigs
Total Protein	Report result	(b) (4)	(b) (4)
(b) (4)	Report result	(b) (4)	(b) (4)
Purity	(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)	N/A

(b) (4), IU: Infections Units, WT: Wild Type, VSV: Vesicular Stomatitis Virus; *Extractable volume for Lot (b) (4) was (b) (4) which is below the specification (b) (4) 1.0 mL). An investigation was initiated which included re-testing on (b) (4) vials. The completed investigation concluded that there was no impact in the quality and safety of the product.

Table 80: Release Testing Results for Clinical Drug Product Lots Manufactured at Merck (b) (4) with (b) (4) (these lots were used in the clinical studies (lot-to-lot consistency) to support the licensure)

Test	Specification	WL00060577	WL00060666	WL00061283
Sterility	No growth of bacteria and fungi detected	No growth of bacteria and fungi detected	No growth of bacteria and fungi detected	No growth of bacteria and fungi detected
Bacterial Endotoxins	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Potency	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Identity	Confirm rVSVΔG-ZEBOV- GP	Identity of VSVΔG-ZEBOV-GP confirmed	Identity of VSVΔG-ZEBOV-GP confirmed	Identity of VSVΔG-ZEBOV-GP confirmed
Test for identity/Potency ((b) (4))	Presence of Filovirus protein	Filovirus protein present	Filovirus protein present	Filovirus protein present
Total DNA Content	Report result	<5 ng/mL	<5 ng/mL	<5 ng/mL
Residual Benzonase	Report result	0.48 ng/mL	0.48 ng/mL	0.53 ng/mL
Physical Appearance	Clear to slightly turbid and colorless to slightly yellow solution	Conforms	Conforms	Conforms
(b) (4)	Report result	(b) (4)	(b) (4)	(b) (4)
Extractable Volume	(b) (4) 1.0 mL	(b) (4)	(b) (4)	(b) (4)
(b) (4)	Report result	(b) (4)	(b) (4)	(b) (4)
General Safety Testing (Mice)	No toxicity observed in mice	Conforms	Conforms	Conforms
General Safety Testing (Guinea pigs)	No toxicity observed in guinea pigs	Conforms	Conforms	Conforms
Total Protein	Report result	2.1 mg/mL	2.1 mg/mL	2.1 mg/mL
(b) (4)	Report result	(b) (4)	(b) (4)	(b) (4)
(b) (4)	Report result	(b) (4)	(b) (4)	(b) (4)

(b) (4), IU: Infectious Unit, WT: Wild Type, VSV: Vesicular Stomatitis Virus

The Drug Product lots listed below were manufactured for (b) (4) Process Performance Qualification at MSD (b) (4).

(b) (4)

The release testing specifications and results for the Engineering and Drug Product lots manufactured for Process Performance Qualification at MSD (b) (4) are provided below.

Table 82: Release Testing Results from (b) (4) PPQ Drug Product Lots Manufactured at MSD

Test		Specification	(b) (4) (b) (4)	Lot	PPQ Lot (b) (4)	PPQ Lot ^{(b) (4)}	PPQ Lot ^{(b) (4)}	PPQ Lot ^{(b) (4)}
Sterility		No growth	No growth		No growth	TBT	TBT	TBT
Bacterial Endotoxins		(b) (4)	(b) (4)		(b) (4)	TBT	TBT	TBT
Potency		(b) (4)	(b) (4)		(b) (4)	TBT	TBT	TBT
Identity		Confirm rVSVΔG-ZEBOV-GP	Confirmed rVSVΔG-ZEBOV-GP		Confirmed rVSVΔG-ZEBOV-GP	TBT	TBT	TBT
Physical Assessment	Color	(b) (4)	(b) (4)		(b) (4)	TBT	TBT	TBT
	Opalescence ^a	(b) (4)	(b) (4)		(b) (4)	TBT	TBT	TBT
	Particulates	No visible particulates	No visible particulates		No visible particulates	TBT	TBT	TBT
(b) (4)	(b) (4)	(b) (4)	(b) (4)		(b) (4)	TBT	TBT	TBT
Extractable Volume		The (b) (4) volume of (b) (4) containers is no less than (b) (4)	(b) (4)		(b) (4)	TBT	TBT	TBT
(b) (4)	(b) (4)	(b) (4)	(b) (4)		(b) (4)	TBT	TBT	TBT
Total Protein		(b) (4)	(b) (4)		(b) (4)	TBT	TBT	TBT

^aAt time of (b) (4) batch, opalescence specification was (b) (4); NR: Test Not Required; TBT: To Be Tested.

3.2.P.5.5 Characterization of Impurities

Process-related impurities and DS-related impurities have been described in 3.2.S.3.2 Impurities. BDS impurities include (b) (4). Sufficient clearance of all process impurities is obtained during the manufacturing process ((b) (4)). Process related impurities from the Bulk Drug Substance manufacturing process are discussed in section 3.2.S.3.2 Characterization – Impurities.

Residual Host Cell DNA and Residual Benzonase tests are performed on (b) (4), but the acceptance criteria are applied to the final filled container by calculation see section 3.2.P.3.5.3 Process Validation and/or

Evaluation (PPQ Only Tests). No additional impurities are introduced in the Formulation and Filling Process of Ebola Zaire Vaccine.

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

The information provided is acceptable. All DP specifications were met. Release testing results support consistency of product manufacture. Impurities are adequately controlled.

3.2.P.6 Reference Standards or Materials

No reference standards are used in testing the Drug Product.

3.2.P.7 Container Closure System

The Ebola Zaire Vaccine DP container closure system consists of a (b) (4) borosilicate clear glass tubing vial, a 13 mm chlorobutyl stopper and a 13 mm flip-off aluminum seal. The vial primary packaging is provided in the table below. Primary packaging components may be accepted based on supplier Certificate of Analysis. Each lot of components received are inspected for defects, and dimensional checks may also be performed.

Table 83: Primary Packaging Component Summary

Component	Description	Compendial Compliance
Vial	2.0 mL - (b) (4) borosilicate clear tubing glass vial, 13 mm finish	(b) (4)
Vial Stopper	13 mm (b) (4) coated (b) (4) elastomer	(b) (4)
Cap/Seal	13 mm, aluminum seal with dark red plastic flip-off cap	N/A

N/A: not applicable - no product contact.

The glass vials and the vial stopper were previously submitted to the FDA and reviewed under Master File (MF) (b) (4) and MF (b) (4), respectively. Letters of authorization are provided in this BLA.

Labels are affixed to the filled and capped vials. Ten vials are inserted into opaque cartons, along with a package insert. The lot number and expiry date are applied to both the labels and cartons.

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

The information provided is acceptable. The DP container was assessed for extractables and leachables in appropriate studies conducted under MF (b) (4) and MF (b) (4) and found to be adequate. The DP container closure system is safe for its intended use.

3.2.P.8 Stability

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

The proposed expiry period for the Ebola Zaire Vaccine DP manufactured at MSD (b) (4), is 36 months for product stored at -70±10°C. An expiry extension to up to (b) (4) is planned to be filed when real time stability data from (b) (4) or more historical lots become available. A summary of the stability protocol that is used to monitor the DP PPQ batches at the long-term storage condition is provided below.

Table 84: Stability Study Protocol for PPQ DP Batches Stored at -70±10°C

Tests	Method References	Time Points (months)	Acceptance Criteria
Physical Assessment	3.2.P.5.2.5 Analytical	0, 3, 6, 9, 12, 18,24, 36, (b) (4)	Opalescence: (b) (4) (b) (4)

	Procedures - Physical Assessment		Color: (b) (4)
			Particulates: No visible particulates
(b) (4)	(b) (4)	(b) (4)	(b) (4)
Potency	3.2.P.5.2.3 Analytical Procedures – Potency	0, 3, 6, 9, 12, 18, 24, 36, (b) (4)	(b) (4)
Sterility	3.2.P.5.2.1 Analytical Procedures – Sterility	0, 12, 24, 36, (b) (4)	No growth
Container Closure Integrity	3.2.P.5.2.10 Analytical Procedures – CCI	0, 12, 24, 36, (b) (4)	Pass

Stability studies at accelerated temperature conditions for DP PPQ batches will be also conducted for the full-scale manufacturing demonstration batches. The accelerated stability studies will be performed at (b) (4). Full scale manufacturing three DP PPQ batches will be placed on stability at (b) (4) for characterization purpose. The stability protocol and acceptance criteria at (b) (4) are provided below.



Since no stability data are available for the DP PPQ batches manufactured at MSD (b) (4), and because these stability data will be only available post-approval, the sponsor provided a supplemental information on the stability testing at $-70\pm 10^{\circ}\text{C}$ and at accelerated temperature conditions (b) (4) performed at (b) (4), on the Ebola Zaire Vaccine DP batches intended for use in clinical trials in single dose vials (manufactured at (b) (4)) and DP batches intended for use as emergency supplies in 10-dose vials (manufactured at (b) (4)). The information includes protocols, acceptance criteria, and results for the (b) (4) DP batches

are provided in this BLA. A total of (b) (4) DP batches from (b) (4) and (b) (4) DP batches from (b) (4) have been initiated on stability studies at the frozen condition (-70±10°C), see below. The methods used to evaluate the stability of the DP batches were selected with the intent of ensuring the quality of the vaccine with respect to container closure integrity, potency and general quality characteristics. The methods used include appearance, (b) (4), potency, bacterial endotoxin, container closure integrity (CCI), and sterility.

It should be noted that the (b) (4) stability samples were filled in 2.0 mL - (b) (4) glass vial with 13 mm bromobutyl rubber stopper, while commercial vial samples were filled as (b) (4) glass vial with 13 mm chlorobutyl stopper with (b) (4) coating. The same vials were used but the stopper and coating were different. As the vial samples were stored frozen, the difference in stoppers is not expected to have a negative impact on the product stability profile. The DP batches from (b) (4) were manufactured from the (b) (4) and vialled in the (b) (4) format ((b) (4) glass vial, 20 mm chlorobutyl stopper with (b) (4) coating). The stability data generated on the (b) (4) format are also provided to support the single dose format. Tables below provide a summary of the stability batches tested at (b) (4) at respective temperature conditions.

Table 88: List of Drug Product Stability Studies Tested at (b) (4)

Batch number	Manufacture Site	Manufacture Date	Stability Sample Storage Site	Stability Study Conditions
(b) (4)	(b) (4)	(b) (4)	(b) (4)	-80°C
				-80°C, (b) (4)
				-80°C, (b) (4)
				-80°C, (b) (4)
				-80°C
				-80°C, (b) (4)
				-80°C, (b) (4)
				-80°C
	Merck (b) (4)		Merck (b) (4)	-70°C, (b) (4)
	Merck (b) (4)		Merck (b) (4)	-70°C
	Merck (b) (4)		Merck (b) (4)	-70°C, (b) (4)
	Merck (b) (4)		(b) (4)	-80°C, (b) (4)

(b) (4)

Table 89: List of Drug Product Stability Studies for the (b) (4) Format

Batch number	Manufacture Site	Manufacture Date	Stability Study Conditions
(b) (4)	(b) (4)	(b) (4)	-70°C, (b) (4)
(b) (4)	(b) (4)	(b) (4)	-70°C, (b) (4)
(b) (4)	(b) (4)	(b) (4)	-70°C, (b) (4)

The stability data for these batches available to date (i.e., for 36^{(b) (4)} months) at this condition ((b) (4) - 70±10°C) support the proposed long-term storage condition (-70±10°C) and expiry dating. All studied parameters met the acceptance criteria. All stability testing for the batches at long-term storage condition (-70±10°C) have been and will continue to be performed at (b) (4) until the studies are complete. The completed stability study results will be provided post approval.

In addition to the studies at the long-term storage condition, a subset of the (b) (4) DP batches was evaluated for stability at accelerated conditions, including (b) (4). The protocols, acceptance criteria, and results for the (b) (4) DP batches tested at accelerated conditions are provided in this BLA (see Section 3.2.P.8). Data generated at the accelerated conditions are for characterization purposes as well as to support potential product handling at (b) (4) temperature conditions.

For studies at (b) (4), there were no changes in appearance or container closure integrity for any of the batches monitored. No significant change in (b) (4) was observed. Potency data generated to date from respective stability study batches stored at (b) (4) indicated that the product batches experienced some loss of potency. Therefore, the available potency data for the DP lots on the stability indicated that the product is stable and is not expected to incur significant potency loss at the proposed long-term storage condition (-70±10°C). Overall, the estimated potency loss rate for the (b) (4) conditions are summarized in the table below.

(b) (4)

Using the estimated loss rates, the calculated total loss of potency was (b) (4) when the product would be exposed to the (b) (4) temperature conditions (b) (4). To account for the expected potency loss when thawed product is exposed to (b) (4) temperature conditions, it is appropriate to raise the minimum release specification for the product. The minimum release specification has been calculated as (b) (4) based on the expiry specification of 7.2×10^7 pfu/mL at the end of expiry period of 36 months. Data from the clinical studies have shown that the vaccine is immunogenic and protective at a dose of 7.2×10^7 pfu/mL (see below), and therefore, a minimum release potency specification of 7.2×10^7 pfu/mL is acceptable. The vaccine lots administered in the clinical trials along with potency testing results are provided in the table below.

Table 91: V920 Vaccine Lots and Potencies from Clinical Trials

V920 DP Lot	Clinical Trial(s) ^a	Nominal Dose (Target dose)	Actual Potency with (b) (4) Method: Used for Clinical Trials	Actual Potency with (b) (4) Method: Used for Commercial Lot Release
003 05 13 (b) (4)	V920-001 through V920-009 inclusive	1.0×10^8 pfu/mL administered undiluted or diluted as appropriate to reach target doses in dose-ranging studies ^c	1.08×10^8 pfu/mL	5.3×10^8 pfu/mL ^b
001 10 14 (b) (4)	V920-009 V920-010 V920-011	1.0×10^8 pfu/mL (b) (4) = 2.0×10^7 pfu/mL	2.4×10^8 pfu/mL (b) (4) = 4.8×10^7 pfu/mL	3.6×10^8 pfu/mL ^b (b) (4) = 7.2×10^7 pfu/mL
001 10 14 (b) (4)	V920-012	1.0×10^8 pfu/mL	2.4×10^8 pfu/mL	3.6×10^8 pfu/mL ^b
WL00060577 (Merck (b) (4) lot)	V920-012	2.0×10^7 pfu/mL	6.6×10^7 pfu/mL dose	N/D
WL00060666 (Merck (b) (4) lot)	V920-012	2.0×10^7 pfu/mL	6.6×10^7 pfu/mL dose	N/D
WL00061283 (Merck (b) (4) lot)	V920-012	2.0×10^7 pfu/mL	5.4×10^7 pfu/mL dose	N/D

^aClinical trials V920-009 and V920-012 used more than one V920 lot each. ^bThese results are from retest using the (b) (4) method that will be used to release commercial lots. ^cThe nominal doses prepared by (b) (4) of the released drug product and used in the clinical trials ranged from 3×10^3 pfu/mL to 1×10^8 pfu/mL. N/D – not done.

Finally, the available stability data for the DP (including the clinical lots used), along with the evaluation of potency stability data, support the proposed expiry period of 36 months at the long-term storage of $-70\pm 10^{\circ}\text{C}$ and the time allowances of 2 weeks at $2-8^{\circ}\text{C}$ and 4 hours at 25°C after the product is thawed.

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

Stability data have been collected on the Ebola Zaire Vaccine DP batches as presented above. Stability studies for the DP PPQ lots manufactured at MSD (b) (4) will be continued as described until completion. In addition, a routine (b) (4) stability program is proposed. The (b) (4) program will monitor at least (b) (4) DP batch per production (b) (4) for a minimum of 36 months at the long-term storage condition ($-70\pm 10^{\circ}\text{C}$). The table below provides a summary of the protocol to be used for routine (b) (4) stability monitoring. The routine (b) (4) stability protocol is intended to be used for future stability studies to support commercial product manufacture. The (b) (4) stability protocol will effectively assess the stability of the vaccine throughout the proposed expiry period of 36 months.

Table 92: Routine Annual Stability Protocol for Drug Product ($-70\pm 10^{\circ}\text{C}$)

Test	Method References	Test Intervals (months)*
Physical Assessment	3.2.P.5.2.5 Analytical Procedures - Physical Assessment	0, 6, 12, 24, 36
(b) (4)	3.2.P.5.2.6 Analytical Procedures – (b) (4)	(b) (4)
Potency	3.2.P.5.2.3 Analytical Procedures – Potency	0, 6, 12, 24, 36
Sterility	3.2.P.5.2.1 Analytical Procedures – Sterility	0, 36
Container Closure Integrity (CCI)	3.2.P.5.2.10 Analytical Procedures – CCI	0, 12, 24, 36

*Additional samples will be pulled at the expiry interval. Samples will be thawed and stored for 2 weeks at $5\pm 3^{\circ}\text{C}$ and 4 hours at 25°C prior to testing.

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

The information provided is acceptable. Available interim stability results for DP batches manufactured at (b) (4) and stored at $-70\pm 10^{\circ}\text{C}$ met acceptance criteria and provide further evidence to support the proposed long-term storage condition at this storage conditions. These stability studies are still in the progress and the final results for DP batches manufactured at (b) (4), and for (b) (4) PPQ DP batches will be available post-approval.

However, several deficiencies were identified in this section. They were addressed and resolved successfully through a request for additional information from the sponsor. The responses provided by the sponsor in amendment 35 are considered acceptable (see below).

Agency Question:

Regarding the justifications for DP release potency and stability acceptance criteria:

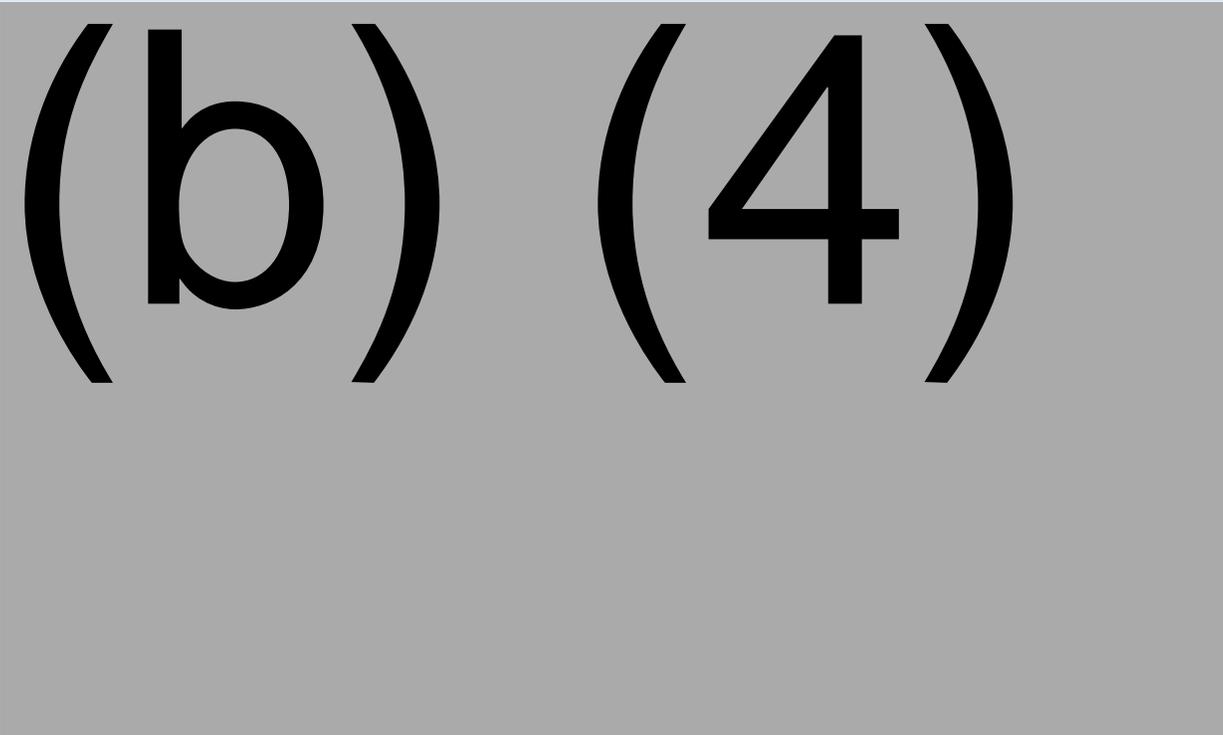
- The lower limit of 7.2×10^7 pfu/mL for stability corresponds to the lower potencies of doses tested in Phase 1, 2 and 3 studies of lots produced and released by (b) (4). Those lots were determined to contain 2.7×10^7 pfu per 1 mL dose when tested for release by (b) (4). However, during product development at Merck, it was determined that those same lots had a potency of 7.2×10^7 pfu per 1 mL dose when tested at Merck’s QC labs using the (b) (4) validated assay. As the stability lower limit (7.2×10^7 pfu/mL) is based on results from Merck’s QC labs, in section 3.2.P.5.6, please include a summary of comparability results from (b) (4) and Merck on the potency of the clinical lots used to justify the lower stability limit.
- You indicate that the release upper limit of (b) (4) is supported by a clinical maximum. In section 3.2.P.5.6, please include information on the clinical study(ies) conducted with this dose.
- We suggest that the relevant clinical lots and doses used to justify the potency lower and upper limits be listed in tabular format showing pertinent study details as provided in section 5.2 Tabular Listing of all Clinical Studies.

Company Response:

a. As described in section 3.2.P.5.6, the commercial potency specification strategy involved the direct retest of the clinical lots manufactured by (b) (4) in the (b) (4) method and incorporated post-release losses into the acceptance criteria. Following the retest of the (b) (4) clinical lots in the (b) (4) potency assay, the minimum release specification was revised to (b) (4). 7.2×10^7 pfu/mL was the clinical minimum therefore the stability specification and label claim were set to this value. The potency results for the lowest (efficacy) and highest (safety) clinical lots are summarized in the table below and the derivation of the commercial potency specifications is show in the Figure below.

(b) (4)

Figure: Derivation of the Commercial Drug Product Potency Specifications



b. As shown in section 3.2.P.5.4, lot 003 05 13 was used in Protocols 1 through 9 and was the batch used to establish the upper specification. This information was added to section 3.2.P.5.6.

c. A summary of all clinical lots and associated protocols is located in section 3.2.P.5.4. The relevant batches (001 10 14 and 003 05 13) used to set the specifications are included in section 3.2.P.5.6 and a link has been added to refer to section 5.2 for the study details.

3.2.A APPENDICES

3.2.A.1 Facilities and Equipment

I did not review this section, except for specific equipment which comes into contact with the (b) (4) DP such as container closure systems and filtration systems.

3.2.A.2 Adventitious Agents Safety Evaluation

See section 3.2.S.2.3 above for assessment of materials of biological origin in the (b) (4) used during manufacture of Ebola Zaire Vaccine were rigorously tested, using validated methods, to provide high confidence that extraneous agents are not present in the DP. (b) (4)

No raw material of human origin has been used in any manufacturing stage of (b) (4) DP, however, (b) (4) raw materials, (b) (4) Benzonase, (b) (4) include (b) (4) derived ingredient which was used during manufacture of the incoming raw material. (b) (4)

Benzonase is an enzyme derived from (b) (4)

The safety of the vaccine with regard to viral and non-viral contamination has been assessed through three different approaches: (b) (4)

Viral Clearance Studies

There is no dedicated viral inactivation or clearance step in the manufacturing process as the vaccine is a live vaccine.

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

The information provided is acceptable.

3.2.A.3 Novel Excipients

Rice-derived recombinant Human Serum Albumin (rd-rHSA) is used as a stabilizer component in the Ebola Zaire Vaccine. The rd-rHSA is a novel excipient, as such full details of manufacture, characterization and controls for the rd-rHSA is provided in the BLA. rd-rHSA is a plant-derived raw material sourced from a third-party supplier. The sites responsible for manufacturing and testing of rd-rHSA are provided below.

Table 93: Manufacturing and Testing Sites for rice-derived recombinant Human Serum Albumin

Name and Address of Site	Responsibility
(b) (4)	

(b) (4)

Manufacturing Process and Process Controls

(b) (4)

(b) (4)

(b) (4)

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

The information provided is acceptable. Based on the available data, the rd-rHSA is a safe excipient for formulation of this vaccine.

3.2.R Regional Information (USA)

□ Executed Batch Records

The executed batch records for (b) (4) completed (b) (4) and (b) (4) completed PPQ (b) (4) batches, and (b) (4) completed (b) (4) DP batch were reviewed during the pre-approval inspection at (b) (4); see Establishment Inspection Report for details). Additional executed batch records for PPQ DS and PPQ DP lots as well as non-PPQ lots (b) (5), (b) (7)(E).

□ Method Validation Package

Method validation protocols and reports were reviewed and discussed in sections 3.2.S.4.2.and 3.2.S.4.3 Analytical Procedures and Validation of Analytical Procedures for Drug Substance and 3.2.P.5.2 and 3.2.P.5.3 for Drug Product. All submitted data were reviewed and found to be acceptable.

Overall Reviewer's Assessment of Combination Products Section:

The information provided is acceptable.

□ Comparability Protocols

Not applicable

Other eCTD Modules

Module 1

A. Environmental Assessment

An environmental assessment (EA) in accordance with 21 CFR 25 that evaluates potential environmental impacts due to use and disposal of this product has been prepared and provided in section 1.12.14. An environmental assessment is needed for this product because rVSVΔG ZEBOV GP is considered to be a Genetically Modified Organism (GMO). This EA has been prepared in accordance with FDA's Guidance for Industry on "Determining the Need for and Content of Environmental Assessments for Gene Therapies, Vectored Vaccines, and Related Recombinant Viral or Microbial Products (March 2015)". V920 is not intended for dispersal in the environment at large, but for direct administration by intramuscular injection into people to be vaccinated against Zaire Ebola virus.

The primary concern with shedding of live virus vaccines is transmission to potentially susceptible individuals and animals. The applicant stated in the BLA that transmission of the V920 vaccine virus through close contact with livestock is accepted as a theoretical possibility, and vaccine recipients should attempt to avoid exposure of livestock to blood and bodily fluids for at least 1 month following vaccination. The V920 vaccine virus shedding of (measured by the detection of rVSV RNA in saliva, urine, and fluid from skin vesicles) was observed in adults

but the V920 vaccine virus transmission (human-to-human or human-to-animal) was not investigated in the clinical studies.

A summary of the virulence and the pathogenicity potential of wild type (wt)-VSV and V920 in relevant species are provided below (see table 95 and 96). Therefore, based on the data provided in the environmental assessment report (see section 1.12.14) and clinical data on limited shedding of the vaccine virus in adults, the results of the biodistribution and persistence in non-human primates, and lack of horizontal transmission of the vaccine virus in pigs demonstrated in an experimental setting, it could be concluded that the V920 vaccine strain has negligible potential environmental impact. The vaccine is restricted for authorization of administration, storage and disposal to trained medical personnel. Any unused vaccine or waste material should be disposed of in accordance with local requirements and ensure medical waste and other cleaning materials do not come in contact with livestock.

Reviewer’s Assessment: *the environmental assessment provided by the applicant is adequate. The data on the vaccine virus viremia and shedding in vaccinees were reviewed by the clinical reviewer on this BLA and were adequately addressed on the product label.*

Table 95: Summary of the virulence potential of wt-VSV and V920 in relevant species

Species	wt-VSV	V920
Rodents	Consistently develop a detectable viremia following VSV-NJ and VSV-I infection; viral titers in mouse brain for rVSV-wt (4x10 ⁵ pfu/g, 2 days post inoculation).	Strongly attenuated versus wild type, ca. 1.000-10.000-fold more attenuated compared to wild-type VSV; not detectable under same experimental condition for rVSVΔG-ZEBOV-GP virus.
Livestock	Significant disease in pigs, cattle, and horses.	rVSVΔG-ZEBOV-GP is strongly attenuated in swine (1.000- 10.000-fold). No information is available for cattle and horses.
Arthropods	Carriers, able to eliminate virus by autophagy prior to virus replication	No replication in arthropods tested, no transmission detected.
Non-human primates	Neurovirulent, only when administered intrathalamically.	rVSVΔG-ZEBOV-GP is not neurovirulent. Well tolerated, low to moderate level viremia, replication effectively controlled in immunosuppressed NHP models.
Humans	Infrequent transmission to humans from livestock in VSV endemic areas; rarely capable of replicating, self-limiting infection.	Low to moderate level viremia.

Table 96: Summary of the pathogenicity potential of wt-VSV versus V920 relevant species

Species	wt-VSV	V920
Rodents	Highly pathogenic; lethal	Apathogenic in all administration routes.
Livestock	Causes acute but mild and transient disease with symptoms of blistering and ulcerations of the mucosa and dermis of mouth, teats and hoofs; disease resolves spontaneously	Deletion of VSV-G from virus construct as in V920 does not significantly attenuate or ablate clinical signs in swine. No information is available for cattle and horse.
Arthropods	Carriers, life span does not seem to be affected.	No replication in arthropods tested, life span not tested.
Humans	Mild flu-like disease with fever, myalgia, headache, occasional blistering in the pharynx; self-limiting and transient; rare, even in enzootic areas	Ongoing clinical trials had some reports of flu-like symptoms (including fever, myalgias, chills, headache, fatigue, abdominal pain, nausea, vomiting, and diarrhea) 1-3-day post-infection. Transient decreases in white blood cell and platelet counts observed in some subjects starting 1-3 dpi (resolved within 2 to 4 days). Some reports of arthralgia and/or arthritis and skin lesions 1-2 weeks post vaccination. Most events were mild to moderate resolved spontaneously within several days or weeks.

B. Labeling Review

Full Prescribing Information (PI):

I reviewed and commented on the product-related sections of the PI listed below. Please see the approved PI for information on the following sections:

- Dosage Forms and Strengths
- Description
- Clinical Pharmacology/Mechanism of Action
- How Supplied
- Storage and Handling

Reviewer's Assessment: The above sections of the Full Prescribing Information are acceptable.

Modules 4 and 5

Analytical Procedures and Validation of Analytical Procedures for Assessment of Clinical and Animal Study Endpoints

Immunological and virological assays were used to assess the vaccine performance. Assays that have been used in support of primary endpoints and/or have been used consistently throughout clinical development, are the following:

- Ebolavirus (Zaire) IgG Enzyme Linked Immunosorbent Assay (ELISA)
- rVSV-ZEBOV-GP Plaque Reduction Neutralization Test (PRNT)
- (b) (4) Ebolavirus RT-PCR

Ebolavirus (Zaire) IgG Enzyme Linked Immunosorbent Assay (ELISA)

The ELISA has been developed, successfully validated and previously reviewed under MF (b) (4).

Principle: The ELISA for the quantitation of human IgG antibodies which bind to ZEBOV GP. (b) (4)

The validation results of this ELISA demonstrate that the assay is suitable for its intended use, i.e., for the analysis of human serum samples for the presence of IgG antibodies to *Zaire ebolavirus* GP (for details, see MF (b) (4)). The clinical significance of IgG antibodies to *Zaire ebolavirus* GP measured in this ELISA is unknown. However, the levels of antibody titers were evaluated in the clinical trials to assess the humoral response to the vaccination.

The Ebolavirus (Zaire) IgG ELISA was fully validated for the assessment of antibody responses in clinical and non-clinical studies in non-human primates. The following parameters were assessed using reference standards and incurred samples: LOD, LOQ, dilutional linearity, intermediate precision, repeatability, specificity, interference, selectivity (see MF (b) (4) for details). All parameters met their acceptance criteria.

Reviewer's Assessment: The parameters and validity criteria selected for the validation studies are adequate and were reviewed by the statistician for the clinical assays assigned on this BLA. The ELISA assay is suitable for its intended use.

rVSV-ZEBOV-GP Plaque Reduction Neutralization Test (PRNT)

Principle: The PRNT can be used to determine the neutralizing antibody levels in serum following the administration of the vaccine, rVSVΔG-ZEBOV-GP. In this test, (b) (4)

(b) (4) Determination of the (b) (4) percent neutralizing titer (PRNT^{(b) (4)}) is based upon the percent reduction in viral plaques in the presence of serum compared to that of the virus control without serum and is calculated by linear regression. It should be noted that this PRNT was not evaluated in comparison to a PRNT, which uses a wild-type *Zaire ebolavirus*, and which should be performed under BSL-4 conditions. The PRNT validation results confirm the operating and performance characteristics of the PRNT assay and demonstrate that the assay is suitable for its intended use. Clinical significance of neutralizing antibodies to rVSVΔG-ZEBOV-GP vaccine virus measured using this PRNT is unknown. However, the levels of antibody titers were evaluated in the clinical trials to assess the humoral response to the vaccination.

The Ebolavirus (Zaire) PRNT was fully validated for the assessment of antibody responses in clinical and non-clinical studies in non-human primates. The following parameters were assessed using reference standards and incurred samples: LLOQ, ULOQ, dilutional accuracy/linearity, intermediate precision, repeatability, specificity, interference (see section 5.3.1.4. for details). All parameters met their acceptance criteria.

Reviewer's Assessment: The parameters and validity criteria selected for the validation studies are adequate and were reviewed by the statistician for the clinical assays assigned on this BLA. The PRNT assay is suitable for its intended use.

(b) (4) Ebolavirus RT-PCR for the detection of Ebola virus disease (EVD)

The (b) (4) Ebolavirus RT-PCR Kit was authorized under Emergency Use Authorizations (EUA) by FDA on (b) (4). The test was intended for qualitative detection of RNA from Ebola virus (b) (4)

(b) (4) on specified instruments in (b) (4) plasma from individuals with signs and symptoms of Ebola virus infection in conjunction with clinical and epidemiological risk factors. The (b) (4) Ebolavirus RT-PCR Kit (b) (4) is intended for use only under the Emergency Use Authorization (EUA) by laboratories certified under the Clinical Laboratory Improvement Amendments of 1988, 42 U.S.C. §263a, to perform high complexity tests, or by similarly qualified non-U.S. laboratories, and is limited to clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. Information about this test kit was previously submitted to IND 16131/118 and was resubmitted in amendment 18 of the BLA.

Principle: (b) (4)

(b) (4)

Potential Limitations: The (b) (4) Ebolavirus RT-PCR Kit (b) (4), when used in conjunction with the PCR instruments listed in the Emergency Use Authorization (EUA), appears to have demonstrated adequate performance in non-clinical settings. In the “ring vaccination” clinical study the Guinean national surveillance network identified a few EVD cases using this assay or assays similar to the (b) (4) Ebolavirus RT-PCR Kit (b) (4). After initial identification of cases, the majority of the EVD cases were confirmed by the European Mobile Laboratory (EML) using the (b) (4) Ebolavirus RT-PCR Kit (b) (4) with either (b) (4) (b) (4), which were not listed as approved PCR instruments in the (b) (4) for the kit. The retest results performed by EML showed high positive percent agreement with the primary results by the Guinean national surveillance network. In another experiment [“External Quality Assurance Panel” study], (b) (4) clinical samples were tested in (b) (4) labs using combinations of different PCR assay kits, extraction kits, and PCR instruments. Although the number of samples evaluated in the experiment was small, the results from (b) (4) laboratories, which used the (b) (4) Ebolavirus RT-PCR Kit (b) (4) with the (b) (4) Viral RNA extraction kit and (b) (4) (b) (4) PCR instrument, had reasonable agreement with the results from other laboratories that used different PCR assays, extraction kits, or PCR instruments. Nevertheless, no comparison has been performed for the (b) (4) Ebolavirus RT-PCR Kit (b) (4) when used with the (b) (4) approved PCR instruments and with the (b) (4) (b) (4) PCR instruments (the EML setup). According to an internal discussion, the review team considered that the results from this “External Quality Assurance Panel” study appears to suggest that the EML setup would provide similar performance as the setups with other assay kits and PCR instrument combinations. Thus, the determination of EVD cases in the “ring vaccination” clinical study was likely reliable, given that the majority of the EVD cases were confirmed by the EML.

Overall Reviewer’s Assessment of Relevant Sections of Module 5 (clinical assays):

The information provided is acceptable. The parameters and validity criteria selected for the validation studies are adequate and were reviewed by the statistician for the clinical assays assigned on this BLA. Validation results assure that methods used are suitable for their intended purpose.

Several deficiencies were identified in this section. They were addressed and resolved successfully through a request for additional information from the sponsor. The responses provided by the sponsor in amendments 51 and 55 are considered acceptable (see below).

Agency comment:

Regarding the rVSV-ZEBOV-GP Plaque Reduction Neutralization Test (PRNT), the (b) (4) percent neutralizing titer (PRNT (b) (4)) is based upon the percent reduction in viral plaques in the presence of serum compared to that of the virus control without serum and is calculated by linear regression. Please clarify why the (b) (4) percent neutralizing titer (PRNT (b) (4)) was selected as a reportable titer.

Company response:

The rationale for selecting a PRNT (b) (4) assay endpoint was based on the assay qualification data, which demonstrated that total assay precision was improved compared to a PRNT (b) (4) endpoint with no significant loss or decrease in assay sensitivity. Therefore, the PRNT (b) (4) assay endpoint dilution was applied in the formal validation experiments where all prospectively defined assay suitability criteria were successfully met.

Agency comment:

Regarding the clinical assay(s) to measure viremia and shedding, we note that viremia and shedding were assessed in several Phase 1/2 clinical studies and results of these assessments are described in the ERVEBO package insert (PI). Please provide the RT-PCR method used to test viremia and shedding in samples from these studies and available qualification information, including the limit of detection (LOD) and lower limit of quantification (LLOQ). In addition, please specify the laboratory site(s) where these test(s) were conducted.

Company response:

In the V920 Phase 1 clinical program, subjects in the blinded and open-label trials were assessed for vaccine viremia (detection of the V920 in the blood) and viral shedding of V920 postvaccination using real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assays. The RT-PCR assays were conducted by different laboratories of external partners, and the assay methods were not standardized across the program.

V920-001 and V920-002

The RT-PCR assay for the V920-001 and V920-002 trials was a qualitative assay conducted at the Walter Reed Army Institute of Research (WRAIR), Silver Spring, Maryland. The method used the (b) (4) RNA extraction kit and the (b) (4) rRT-PCR (b) (4) Assay version (b) (4) (14-AUG-2014) [(b) (4)]. Valid test run criteria required (b) (4)

Ct values for subject specimens subjected to (b) (4) rRT assay were indicative of VSV viremia (plasma) or VSV in saliva or urine specimens. Specimens with no Ct value by (b) (4) rRT-PCR assay, but with a Ct value by human RNase P rRT-PCR assay were scored as virus not detected.

V920-003

The RT-PCR assay for the V920-003 trial was a quantitative assay conducted at (b) (4) . Real-time reverse-transcriptase polymerase chain reaction (RT-PCR) was performed using the same method developed at WRAIR to identify vaccine rVSV in plasma, saliva or urine. The lower limit of detection was (b) (4) copies/mL for blood and saliva, and (b) (4) copies/mL for urine [see Clinical Study Report V920-003 submitted in amendment 55].

V920-004

The RT-PCR assay for the V920-004 trial was a quantitative assay that was qualified and conducted by (b) (4) . The SOP is provided in document TSOP.119.00762-FDX [see amendment 55]. The procedure used (b) (4) Kit for extraction and (b) (4) chemistry on the (b) (4) Real Time PCR System for amplification and detection. Primer and probes were based on RNA sequences at the junction of the VSV matrix gene and ZEBOV-GP sequences in the vector such that this assay is specific for the V920 vaccine and does not detect wild-type VSV. The limit of detection of viremia by RT-PCR was (b) (4) copies per mL, and it was determined that this corresponded to less than one infectious unit, with the ratio of genome equivalents to plaque forming units being approximately (b) (4)

V920-005

The RT-PCR assay for the V920-005 trial was a quantitative assay conducted by (b) (4) . RT (b) (4) qPCR was performed using (b) (4) RT-PCR Kit ((b) (4)) and (b) (4) targeting the (b) (4) gene for amplification, and (b) (4) for detection [(b) (4)]. The LLOD for the RT-PCR assay to detect vaccine virus was (b) (4) copies/mL. RT-PCR results were deemed quantifiable if the result was (b) (4) copies/mL, the lower limit of quantification (LLOQ).

V920-006

The RT-PCR assay in the V920-006 trial was a quantitative assay that was conducted by (b) (4). The assay was performed using the (b) (4) RT-PCR Kit ((b) (4)) [see Clinical Study Report V920-006 provided in amendment 55, and (b) (4)]. VSV-specific primers targeted the (b) (4) gene. For quantitation, calibration curves were generated using a serial (b) (4)-fold dilution of rVSVΔG-ZEBOV-GP with a maximum range of (b) (4)-log (from (b) (4) copies/mL). Samples with a mean threshold cycle value of (b) (4) were considered positive. Based on the calibration curves, the data were subdivided into 3 groups: 1) quantifiable copies/mL; 2) copies/mL below the limit of quantification (BLQ); and 3) samples with no positive PCR signal, thus not detectable and referred to as below the limit of detection. Samples with (b) (4) copies/mL were grouped into BLQ; the viral load of these samples was estimated to be (b) (4) copies/mL.

V920-007 and V920-008

The RT-PCR assay for the V920-007 and V920-008 trials was a quantitative assay that was conducted by the (b) (4) [see SOP-006, VSV-ZEBOV RT-qPCR provided in amendment 55]. Note that the V920-008 trial only assessed viremia and not viral shedding. The RT-PCR procedure used (b) (4) method followed by (b) (4)

The LLOD for the RT-PCR assay to detect vaccine virus was (b) (4) copies/mL. RT-PCR results were deemed quantifiable if the result was (b) (4) copies/mL, the LLOQ.

***Reviewer's Assessment:** The RT-PCR assays used to demonstrate the vaccine virus viremia and shedding in vaccinees across the Phase 1/2 clinical studies were conducted by different laboratories, which were not standardized across different clinical sites where the Phase 1/2 clinical studies were conducted. The qualification parameters, such as limit of detection (LOD) and lower limit of quantification (LLOQ), including the LODs measured for different tested clinical matrices (e.g., blood, urine, synovial fluid), for these RT-PCR assays used at the different clinical sites were not identical. Therefore, any variation in the percentages of vaccinees with virus viremia and shedding, among different clinical sites cannot be precisely evaluated. Overall, the provided method description and qualification parameters of the RT-PCR assays used to measure the vaccine virus viremia and shedding during Phase 1/2 clinical studies are adequate, and these assays were suitable for their intended use during Phase 1/2 clinical studies. Please note that the measuring of the vaccine virus viremia and shedding in vaccinees during Phase 1/2 clinical studies were conducted as secondary (not as a primary) objectives. The use of a validated assay should be considered for any future clinical studies where the vaccine virus viremia and shedding in vaccinees will be tested (specially in immunocompromised and pediatric populations). I defer this issue to the clinical reviewer on this BLA. Please see follow-up recommendations in introductory item 9.B.1.d List of Post Marketing Agreements (PMAs).*

PHARMACOLOGY STUDIES (SECTION 4.2.1)

Immunogenicity and Efficacy (Challenge) Studies

The preclinical evaluation of this vaccine virus has been studied in a few animal models and has been published (for the published references, see section 2.4 - Nonclinical overview). Immunogenicity and pre-exposure prophylactic efficacy of V920 (or research grade rVSVΔG-ZEBOV-GP vaccine virus), has been demonstrated following a single IM injection in multiple nonclinical studies in rodent species and nonhuman primates (NHP). A single IM injection of rVSVΔG-ZEBOV-GP protected macaques against lethal IM challenge with the virulent *Zaire ebolavirus* strain 42 days after immunization.

The primary pharmacodynamic properties of V920 were evaluated in multiple non-Good Laboratory Practice (non-GLP) immunogenicity and efficacy studies in rodent species and in NHPs. These studies include:

- Evaluation of the prophylactic efficacy of V920 and related candidate vaccines in NHP (published studies);
- Evaluation of the durability of protective immunity (published studies);
- Evaluation of immune correlates (mechanism of action; published studies);
- Evaluation of the immunogenicity and efficacy in NHP in studies conducted at the United States Army Medical Research Institute for Infectious Diseases (USAMRIID), Ft. Detrick, Maryland, USA (see below);
- Evaluation of the immunogenicity of a low dose of V920 (Merck).

Table 97: The USAMRIID Immunogenicity and Efficacy Studies Conducted with V920

Study No.	Type of Study	Species	No. per Group	ROA	Duration of Dosing	Dose Regimen	Findings
(b) (4), (b) (6)	V920 Vaccine Immunogenicity, efficacy	Cynomolgus macaques	8 per treatment group; 3 control animals (27 total animals)	IM vaccination; IM challenge	One dose with 42 days pre-challenge and 28 to 31 days post-challenge; challenge on Day 42	1 mL/dose on Day 0: 3×10^6 , 2×10^7 , or 1×10^8 pfu	Immunogenicity demonstrated in all vaccinated animals, 96% overall survival against lethal ZEBOV challenge
(b) (4), (b) (6)	V920 Vaccine Immunogenicity, efficacy	Cynomolgus macaques	4 to 5 per treatment group; 2 control animals (24 total animals)	IM vaccination; IM challenge	One dose with 42 days pre-challenge and 28 to 36 days post-challenge; challenge on Day 42	1 mL/dose on Day 0: 3×10^2 , 3×10^3 , 3×10^4 , 3×10^5 , or 3×10^6 pfu	Immunogenicity demonstrated in all vaccinated animals, 100% overall survival against lethal ZEBOV challenge
(b) (4), (b) (6)	V920 Vaccine Immunogenicity, efficacy	Cynomolgus macaques	4 to 7 per treatment group; 2 control animals (19 total animals)	IM vaccination; IM challenge	One dose with up to 12 months pre-challenge and 28 to 36 days post-challenge; challenge on Day 42, month 3 or month 12	1 mL/dose on Day 0: 3×10^4 pfu (n=4 in 12-month cohort) or 3×10^6 pfu (all remaining NHP)	Immunogenicity demonstrated in all vaccinated animals, survival against lethal IM ZEBOV challenge was 100% on day 42, 33% at 3 mo. and 43% at 12 mo.

All studies were performed by the USAMRIID under non-GLP conditions. Lot numbers of V920 used in studies are shown in the table below. Challenge dose used in all studies is 1000 pfu. GLP = Good Laboratory Practice; IM = intramuscular; mL = milliliter; mo. = months; No. = number; pfu = plaque-forming unit(s); ROA = route of administration; ZEBOV = *Zaire ebolavirus*

Table 98: Lot Numbers of V920 Used in USAMRIID/Merck Nonclinical NHP Studies

Study	Type of Batch	Source	Lot No.
(b) (4), (b) (6)	Clinical drug product	(b) (4)	003-05-13
(b) (4), (b) (6)	Clinical drug product	(b) (4)	001-10-14
1-year cohort 3-month cohort 42-day cohort	Clinical drug product (b) (4)	Merck Research Laboratories	WL00060557 (b) (4) (b) (4)
Low dose immunization	Bulk Drug Substance	(b) (4)	(b) (4)

Biodistribution Study

In a biodistribution study in cynomolgus macaques, the macaques were administered a single injection of V920 at 2.4×10^8 pfu by the IM route [Section 2.6.7.17C]. The vaccine was well-tolerated, and no findings were observed at each scheduled gross necropsy. The presence of the vaccine virus (VSV nucleoprotein (NP)) in different organs was investigated over a 112-day observation period by Quantitative Reverse Transcription PCR (qRT-PCR). VSV NP RNA-positive tissues were subsequently assayed by plaque assay to determine potential for infectivity. Persistence of vaccine viral RNA was observed primarily in lymphoid tissues by qRT-PCR throughout the duration of the study (up to 112 days). A subsequent plaque assay detected replication-competent virus limited to day 1 post-vaccination, with no evidence of viral replication at any other time point measured (days 7, 56, 84 and 112). Viral RNA after Day 7 was generally confined to tissues lacking potential for shedding in excretions or secretions and showed no evidence of distribution to the brain or spinal cord at any time point.

Reviewer's Note: *The qualification parameters of the RT-PCR assay used in the biodistribution study are not provided in this BLA. Below please see the IR comment to the sponsor and the sponsor's response to it.*

Neurovirulence Study

Published literature from the New England Primate Research Center presents data from a non-GLP study of 21 cynomolgus macaques in which rVSV Δ G vaccines were administered into the brain intrathalamically at 1.0×10^7 pfu/animal (for references please see: PLoS Negl Trop Dis. 2012;6(3):e1567; J Infect Dis. 2007 Nov 15;196 Suppl 2:S404-12). Seven animals received V920, seven received a rVSV Δ G vector expressing the Lake Victoria Marburg virus (MARV) GP, three received wild-type rVSV vector (wt-rVSV), and four received vehicle control. Two of three animals administered with wt-rVSV showed severe neurological symptoms, whereas animals receiving vehicle control, V920, or rVSV Δ G-MARV-GP did not develop these symptoms. Significant neuropathologic changes were observed in the wt-rVSV inoculated animals. However, no significant histomorphological lesions were observed in the neural tissues of any animals from rVSV Δ G-ZEBOV-GP-treated monkeys. These data suggest that rVSV Δ G-ZEBOV-GP vaccine virus lack the neurovirulence properties associated with wild-type VSV. No additional neurovirulence study(ies) were performed by Merck for this vaccine product.

Overall Reviewer's Assessment of Relevant Sections of Module 4 (clinical pharmacology):

The information provided is acceptable. The preclinical evaluation of this vaccine virus has been studied in different animal models, including non-human primates, and has been demonstrated to be safe, immunogenic, and effective.

However, one deficiency was identified in this section. It was addressed and resolved successfully through a request for additional information from the sponsor. The response provided by the sponsor in amendment 55 is considered acceptable (see below).

Agency comment:

Regarding the biodistribution and persistence study in cynomolgus monkeys, we note that RT-PCR was used to show the absence of vaccine virus in tissue samples, including the central nervous system. Please provide the RT-PCR method used to detect the virus in the non-human primate samples from these studies and available qualification information including limit of detection (LOD) and the lower limit of quantification (LLOQ). In addition, please specify the laboratory site where this test was conducted.

Company response:

The NHP biodistribution study and associated RT-qPCR qualification were conducted at the (b) (4) . The RT-qPCR qualification study for detection of

VSV-NP was performed in several different NHP tissue matrices (including CNS). The details of the RT-qPCR assay, including LOD and LOQ, are reported in the PCR qualification report entitled “QD-481 Addendum No. 3, Vesicular Stomatitis Virus (VSV) Nucleocapsid Protein (NP) Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR) Assay Qualification” [see the VSV RT-qPCR Qualification Report provided in amendment 55]. Accuracy, precision, and dilutional linearity were all analyzed to obtain the final variability (CV%) of the assay in each studied tissue matrix. A detailed explanation of formulas used, and data tables obtained during the process of analysis can be found in Appendix 5 of the VSV RT-qPCR Qualification Report. The LODs for each tissue were determined by calculating the geometric mean reportable value at the lowest concentration where there were no false negative results. Since an exact 95% confidence limit could not be calculated, the LOD had to be determined where all measurements (12 replicates) resulted in detection. For all matrices tested in this qualification except synovial tissue, the resulting LOD was equivalent to the LOQ because the lowest input rVSV-ZEBOV-GP concentration with acceptable CV% was also the lowest point with 100% detection. Due to high CV% at the 2.40E+01 and 1.20E+01 PFU/mL input levels for the synovial tissue, the LOQ was calculated using input levels $\geq 1.20E+02$ PFU/mL. However, there were still 12 out of 12 detects at the lower concentrations, allowing for calculation of the LOD at the lowest input concentration (1.20E+01 PFU/mL).

Reviewer’s Assessment: *The method description and qualification parameters of the RT-PCR assay used the biodistribution and persistence study are adequate. The assay was suitable for its intended use.*

UNII assignment: I concur with the list of ingredients for ERVEBO as identified by the Substance Registration System (SRS) team.