

CBER CMC BLA Review Memorandum

BLA STN 125761

AV7909 Anthrax Vaccine Adsorbed, Adjuvanted; CYFENDUS

Anita Verma, Ph.D., Biologist, CBER/OVRR/DBPAP/LRSP
Tod Merkel, Ph.D., Research Microbiologist, CBER/OVRR/DBPAP/LRSP

1. BLA#: STN 125761

2. APPLICANT NAME AND LICENSE NUMBER

Emergent Product Development Gaithersburg, Inc; license #2089

3. PRODUCT NAME/PRODUCT TYPE

CYFENDUS; AV7909 Anthrax Vaccine Adsorbed, Adjuvanted

4. GENERAL DESCRIPTION OF THE FINAL PRODUCT

- a. Pharmacological category: Vaccine.
- b. Dosage form: Sterile suspension, supplied in a multiple-dose vial.
- c. Strength/Potency: Relative Potency in an (b) (4) *Bacillus anthracis* (b) (4)
- d. Route of administration: Intramuscular.
- e. Indication: For post-exposure prophylaxis of disease following suspected or confirmed exposure to *B. anthracis* in persons 18 through 65 years of age when administered in conjunction with the recommended antibacterial regimen.

5. MAJOR MILESTONES

Filing Meeting – 02 June 2022

Mid-Cycle Meeting – 12 January 2023 (canceled by the applicant)

Late-Cycle Meeting – 13 April 2023 (canceled by the applicant)

PDUFA action date – 20 July 2023

6. CMC/QUALITY REVIEW TEAM

Reviewer/Affiliation	Section/Subject Matter
Tod Merkel (TM), OVRP/DBPAP/LRSP	2.3.P Quality Overall Summary Drug Product
	2.4 Nonclinical Overview
	3.2.S.1 through 3.2.S.7
	3.2.P.1 through 3.2.P.8
	4.2.1.1 Nonclinical Study Reports, Primary Pharmacodynamics

Anita Verma (AV), OVRP/DBPAP/LRSP	1.4 Nonclinical overview 1.5 Clinical overview 3.2.S.4.2 Analytical Procedures 3.2.S.4.3 Validation of Analytical Procedures 3.2.P.5.2 Analytical Procedures 3.2.P.5.3 Validation of Analytical Procedures 3.2.P.5.6 Justification of Specification(s) 4.2 (Performance of TNA in nonclinical studies) 5.3 (Performance of TNA in clinical studies)
--------------------------------------	---

7. INTER-CENTER CONSULTS REQUESTED

Reviewer/Affiliation	Section/Topic	In agreement with consult recommendations (Yes/No)
N/A	N/A	N/A

8. SUBMISSION(S) REVIEWED

Date Received	Submission	Comments/ Status
10/12/2021		IR submitted in response to pre-BLA package; reviewed in context of BLA.
12/14/2021	STN 125761/0	Rolling BLA submission
04/19/2022	STN 125761/0.3	Final BLA submission
07/26/2022	STN 125761/0.8	List of differences between AVA and AV7909 DS manufacturing; (b) (4); htpTNA assay
08/17/2022	STN 125761/0.11	Correction to STN 125761/0.8
08/26/2022	STN 125761/0.13	Minor CMC updates
09/21/22	STN 125761/0.18	Response to IR#9 comment 1
09/28/22	STN 125761/0.20	Summary of invalid plates for htpTNA assay for Phase 3 clinical study
11/9/2022	STN 125761/0.25	Minor CMC updates
02/01/2023	STN 125761/0.34	Minor CMC updates
02/10/2023	STN 125761/0.35	Updated stability data; response to IR#21 comment 1
04/03/2023	STN 125761/0.41	Updated stability data Updated response to IR#21 comment 1
04/07/2023	STN 125761/0.44	(b) (4) potency assay
05/10/2023	STN 125761/0.48	Acknowledgement for submission of supplement for (b) (4) potency assay acceptance criteria
05/23/2023	STN 125761/0.50	Discrepancy in percent GCV calculations for (b) (4) revalidation

9. Referenced REGULATORY SUBMISSIONS (e.g., IND BLA, 510K, Master File, etc.)

Submission type and number	Holder	Referenced Item	Letter of Cross-Reference	Comments/Status
BLA 103821	Emergent	DS manufacturing	Yes	Information pertinent to DS manufacturing was reviewed, assessed, and documented in the memo by Tod Merkel in Section 3.2.S.2.

DMF (b) (4)	(b) (4)	CPG 7909 high density polyethylene bottle	Yes	Information pertinent to CPG 7909 container closure was reviewed, assessed, and documented in the adjuvant reviewer's memo.
DMF (b) (4)	(b) (4)	(b) (4) rubber formulation (b) (4)	Yes	Information pertinent to container closure was reviewed, assessed, and documented in the memo by Tod Merkel in Section 3.2.P.2.
DMF (b) (4)	(b) (4)	CPG 7909 polypropylene cap	Yes	Information pertinent to CPG 7909 container closure was reviewed, assessed, and documented in the adjuvant reviewer's memo.
DMF (b) (4)	(b) (4)	Glass vials	Yes	Information pertinent to container closure was reviewed, assessed, and documented in the memo by Tod Merkel in Section 3.2.P.2.
DMF (b) (4)	(b) (4)	CPG 7909 manufacturing	Yes	Information pertinent to CPG 7909 manufacturing was reviewed, assessed, and documented in the adjuvant reviewer's memo.

MF (b) (4)	(b) (4)	Sterile Contract Manufacturing Facility	Yes	Information pertinent to DP manufacturing development and changes was reviewed, assessed, and documented in the facilities reviewer's memo.
IND 14451	Emergent	DP manufacturing development and changes	Yes	Information pertinent to DP manufacturing development and changes was reviewed, assessed, and documented in the memo by Tod Merkel in Section 3.2.P.2.
IND (b) (4)	(b) (4)	Phase 2 Clinical Study	Yes	Information pertinent to DP development submitted to the IND was reviewed, assessed, and documented in the memo by Tod Merkel in Section 3.2.P.2.
MF (b) (4)	NIAID	Non-clinical Animal Studies	Yes	Information pertinent to non-clinical animal studies was reviewed, assessed, and documented in the memo by Tod Merkel in Section 4.2.1.1.
MF (b) (4)	(b) (4)	Bacterial endotoxin testing	Yes	Information pertinent to container closure was reviewed, assessed, and documented in the memo by Tod Merkel in Section 3.2.P.2.

10. REVIEWER SUMMARY AND RECOMMENDATION

A. EXECUTIVE SUMMARY

CMC

Emergent's in-house name for the product is Anthrax Vaccine Adsorbed, Adjuvanted. During development and throughout this submission and review, the product was referred to as AV7909.

The Drug Product (DP) consists of AV drug substance (AV DS) combined with CPG 7909 adjuvant, formaldehyde solution, benzethonium chloride (b) (4) and sodium chloride. The DP is adjusted to volume with water for injection (WFI).

The AV (b) (4) consists of the anthrax vaccine filtrate adsorbed to aluminum hydroxide (Al(OH)₃) and resuspended in a saline preservative solution. The AV (b) (4) is a complex mixture derived from the components present in the media at the (b) (4)

(b) (4), protective antigen (PA). The AV (b) (4) for the firm's licensed product BioThrax® (AVA).

CPG 7909 is a synthetic DNA molecule, 24 nucleotides in length, with a nuclease-resistant phosphorothioate backbone. CPG 7909 binds Toll-like receptor 9 and induces enhanced antigen-specific antibody responses and natural killer T-cell responses. (b) (4)

The biological activity of the DP is due primarily to its induction of antibodies that neutralize the activity of PA. *B. anthracis* virulence requires the action of (b) (4) toxins: (b) (4)

(b) (4)

(b) (4)

DP manufacturing begins with the addition of (b) (4) CPG 7909 (b) (4) with WFI followed by (b) (4). The date of manufacture for AV7909 DP is defined as the date the CPG 7909 adjuvant (b) (4).

Because manufacturing of (b) (4) DP is a continuous process, the allowable (b) (4) for the (b) (4) of formulation of the DP was evaluated during execution of PPQ runs. A maximum (b) (4) to when CPG 7909 is added to initiate formulation of the DP was validated.

The proposed shelf life of AV7909 DP is 48 months when stored at $5\pm3^{\circ}\text{C}$. At the time of final review, (b) (4) of real time stability data was provided for (b) (4).

Results for these (b) (4) lots met all acceptance criteria, supporting the proposed shelf-life of 48 months.

Non-clinical

Due to the rare occurrence of *B. anthracis* infections and the inability to design ethical studies exposing humans to anthrax, conducting clinical-endpoint efficacy studies is not feasible. Therefore, Emergent is pursuing licensure of AV7909 for the post-exposure prophylaxis (PEP) indication under the FDA Animal Rule (21 CFR Part 601, Subpart H, "Approval of Biological Products when Human Efficacy Studies are not Ethical or Feasible").

The ability of PA-based vaccines to generate a robust, protective toxin-neutralizing antibody (TNA) response has been demonstrated in multiple animal models including guinea pigs, rabbits, and non-human primates (NHP). The pathogenic mechanisms resulting in inhalational anthrax are well characterized and have been shown to closely resemble the human disease in the guinea pig, rabbit, and NHP models.

To establish proof-of-concept of AV7909 in a PEP regimen, guinea pigs were challenged via the inhalation route with aerosolized *B. anthracis* (b) (4) spores on Day 0. The animals were treated with ciprofloxacin daily for two weeks and were vaccinated on Days 1 and 8 with AV7909 or a single dose level of AVA. The results demonstrated that AV7909 administered in combination with ciprofloxacin protected guinea pigs from death due to anthrax in a dose-dependent manner and provided a significant added benefit compared to ciprofloxacin treatment alone.

To assess the effectiveness of the proposed AV7909 human dose, immune responses associated with survival in animals were bridged to human immunogenicity data to infer clinical benefit. The TNA assay was used to determine this threshold of protection for AV7909. A validated pan-species serology assay, the high-throughput TNA Assay (htpTNA) was used to assess neutralizing antibody levels induced by AV7909 DP in all non-clinical and clinical studies.

In three guinea pig studies and three NHP studies, groups of animals were immunized with dilutions of AV7909 and were challenged on day 28 or 70 with aerosolized *B. anthracis* spores. Neutralizing antibody (TNA) titers are expressed as a ratio, or 50% neutralization factor (NF₅₀), relative to a reference serum from AVA vaccinated subjects. A 70% probability of survival was associated with TNA NF₅₀ titers ranging from 0.063 to 0.081 in the guinea pig studies and from 0.107 to 0.262 in the NHP studies.

In previous BioThrax Studies, groups of rabbits or NHPs were immunized on Days 0 and 28 with dilutions of BioThrax or placebo and challenged on Day 70 with aerosolized *B. anthracis* spores. A pre-exposure TNA NF₅₀ level of 0.56 corresponded to a 70% probability of survival in rabbits, and a pre-exposure TNA NF₅₀ level of 0.29 corresponded to a 70% probability of survival in NHPs.

Emergent selected the most conservative protective target, an NF₅₀ threshold derived from any of the animal studies conducted with BioThrax or AV7909, as the basis for the primary clinical immunogenicity endpoint in the pivotal AV7909 Phase 3 study (Study EBS.AVA.212). The NF₅₀ threshold of 0.56 was derived from the pivotal BioThrax rabbit PEP study (Study 646-N107247).

Subsequently, five additional rabbit pre-exposure prophylaxis studies were performed. These studies showed that TNA NF₅₀ thresholds in the range of 0.19 to 0.29 correlated with 70% rabbit survival. Logistic regression analysis of pooled study data from these AVA-immunized rabbits (n=632) showed a TNA NF₅₀ threshold of 0.24 was associated with a 70% probability of survival. The NF₅₀ value of 0.240 obtained with the pooled rabbit data analysis is consistent with the NF₅₀ value of 0.29 obtained in the AVA NHP study. The results of the five additional rabbit studies suggest that the original rabbit study yielding the 0.564 NF₅₀ threshold overestimated the TNA threshold level.

Therefore, Emergent proposed using the AVA-immunized NHP TNA threshold of protection NF₅₀ level from NHP study 844 (0.294 NF₅₀) as an acceptable bridging endpoint for a proposed phase 3 trial co-primary endpoint, to which CBER agreed.

The non-clinical animal studies demonstrated that AV7909 induced a rapid TNA response that protected a large proportion of animals from death due to inhalation anthrax in a dose-dependent manner. The TNA thresholds of protection were similar between the two animal models and were not impacted by vaccination schedule or challenge time point. These studies provide supportive animal data for AV7909 PEP licensure and support the TNF NF₅₀ thresholds selected to estimate protection in the clinical trials.

The CMC product information and data in this BLA support manufacturing consistency and product quality. We recommend approval of this BLA.

B. RECOMMENDATION


I. APPROVAL

List of DS and DP manufacturing facilities (TM)

Manufacturer	Responsibility
<u>Emergent BioDefense Operations</u> 3500 N. Martin Luther King Junior Blvd. Lansing, MI 48906-9910, USA FEI: 1873886 DUNS: 026489018	<ul style="list-style-type: none"> ▪ Manufacture of AV7909 BDP up to the filling step ▪ In-process control testing ▪ Inspection, packaging, and labeling of DP ▪ Release testing of DP ▪ Disposition of DP ▪ Stability testing of DP (not including Container Closure Integrity Testing, [CCIT], (b) (4) or Sterility)
(b) (4)	<ul style="list-style-type: none"> ▪ Stability testing of DP (only CCIT and (b) (4)) ▪ Alternative site for testing of Aluminum Content in DP (release and stability)
(b) (4)	<ul style="list-style-type: none"> ▪ In-process control testing and release testing (sterility) ▪ Alternative site for Sterility testing of DP (stability) ▪ Manufacture of DP—filling step only

Inspectional follow-up (AV)

(b) (5)



Lot release requirements (TM)

DBPAP and DBSQC developed a lot release protocol with Emergent, containing the agreed upon tests that will be used to report test results for each lot of AV7909 product to be distributed post licensure. The lot release protocol review is provided in the DBSQC memo.

For each lot of AV7909, Emergent will need to submit a protocol and results of the testing they performed, along with samples of the product from each lot, to permit the Agency to perform confirmatory testing.

DBPAP indicated in an email to DBSQC on 18 May 2023 that confirmatory testing is not required and deferred to DBSQC for determination of the final confirmatory testing strategy. Lot release will be performed via protocol review only. DBPAP will perform the review and disposition of results for the potency (b) (4) test) and purity (b) (4) on (b) (4) tests on the lot release protocol.

II. COMPLETE RESPONSE (CR)

N/A

III. SIGNATURE BLOCK

Reviewer/Title/Affiliation	Concurrence	Signature and Date
Tod Merkel, PhD Research Microbiologist CBER/OVRR/DBPAP/LRSP	Concur	
Anita Verma, PhD Biologist CBER/OVRR/DBPAP/LRSP	Concur	
Michael Schmitt, PhD Chief CBER/OVRR/DBPAP/LRSP	Concur	

Jay Slater Director CBER/OVRR/DBPAP	Concur	
---	--------	--

Review of CTD

Table of Contents

3.2.S DRUG SUBSTANCE	3
3.2.S.1.1 - 1.3 Nomenclature, Structure and General Properties	3
3.2.S.2 Manufacture	4
3.2.S.2.1 Manufacturer(s)	4
3.2.S.2.2 Description of Manufacturing Process	4
3.2.S.2.3 Control of Materials	5
3.2.S.2.4 Controls of Critical Steps and Intermediates	7
3.2.S.2.5 Process Validation and/or Evaluation	8
3.2.S.2.6 Manufacturing Process Development	9
3.2.S.3 Characterization	11
3.2.S.3.1 Elucidation of Structure and Other Characteristics	11
3.2.S.3.2 Impurities	12
3.2.S.4 Control of Drug Substance	13
3.2.S.4.1 Specification(s) and 3.2.S.4.5 Justification of Specification(s)	13
3.2.S.4.2 Analytical Procedures and 3.2.S.4.3 Validation of Analytical Procedures	15
3.2.S.4.4 Batch Analyses	17
3.2.S.5 Reference Standards or Materials	18
3.2.S.6 Container Closure System	18
3.2.S.7 Stability	18
3.2.S.7.1 Stability Summary and Conclusion and 3.2.S.7.3 Stability Data	19
3.2.S.7.2 Post-Approval Stability Protocol and Stability Commitment	19
3.2.P DRUG PRODUCT	19
3.2.P.1 Description and Composition of the Drug Product	19
3.2.P.2 Pharmaceutical Development	19
3.2.P.3 Manufacture	24
3.2.P.3.1 Manufacturer(s)	24
3.2.P.3.2 Batch Formula	25
3.2.P.3.3 Description of Manufacturing Process	25
3.2.P.3.4 Controls of Critical Steps and Intermediates	27
3.2.P.3.5 Process Validation and/or Evaluation	29
3.2.P.4 Control of Excipients	29
3.2.P.4.1 Specifications	29
3.2.P.4.2 and 3.2.P.4.3 Analytical Procedures and Validation of Analytical Procedures	30
3.2.P.4.4 Justification of Specifications	30
3.2.P.4.5 Excipients of Human or Animal Origin	31
3.2.P.4.6 Novel Excipients	31
3.2.P.5 Control of Drug Product	32
3.2.P.5.1 and 3.2.P.5.6 Specification(s) and Justification of Specification(s)	32
3.2.P.5.2 and 3.2.P.5.3 Analytical Procedures and Validation of Analytical Procedures	34
3.2.P.5.4 Batch Analyses I	42


3.2.P.5.5 Characterization of Impurities.....	42
3.2.P.6 Reference Standards or Materials.....	43
3.2.P.7 Container Closure System.....	43
3.2.P.8 Stability.....	43
3.2.P.8.1 Stability Summary and Conclusion and 3.2.P.8.3 Stability Data	43
3.2.P.8.2 Post-Approval Stability Protocol and Stability Commitment	45
3.2.A APPENDICES.....	47
3.2.A.1 Facilities and Equipment	47
3.2.A.2 Adventitious Agents Safety Evaluation	47
3.2.A.3 Novel Excipients.....	47
3.2.R Regional Information (USA).....	47
Executed Batch Records	47
Module 1	48
A. Environmental Assessment or Claim of Categorical Exclusion	48
B. Reference Product Designation Request	48
C. Labeling Review.....	48
Full Prescribing Information (PI)	48
Carton and Container Label.....	49
Module 4	49
Analytical Procedures and Validation of Analytical Procedures for Assessment of Clinical and Animal Study Endpoints	49
Serology assay (TNA) review for nonclinical studies	49
Use of htpTNA assay in nonclinical studies in support of AV7909 PEP indication ..	51
Non-clinical Studies	54
Module 5	60
Serology (TNA) assay review for clinical studies	60
Serologic Assay for Anthrax PA.....	60
Review of the performance of htpTNA assay used in clinical studies.....	61

Module 3


3.2.S DRUG SUBSTANCE (TM)

3.2.S.1.1 - 1.3 Nomenclature, Structure and General Properties

(b) (4)

A large rectangular area of the document is redacted with a solid gray fill, covering approximately two lines of text.A large rectangular area of the document is redacted with a solid gray fill, covering approximately two lines of text.A large rectangular area of the document is redacted with a solid gray fill, covering approximately one line of text.A large rectangular area of the document is redacted with a solid gray fill, covering approximately two lines of text.A large rectangular area of the document is redacted with a solid gray fill, covering approximately two lines of text.A large rectangular area of the document is redacted with a solid gray fill, covering approximately six lines of text.A large rectangular area of the document is redacted with a solid gray fill, covering approximately one line of text.

(b) (4)

A large rectangular area of the document is redacted with a solid gray fill, covering approximately one line of text.

(b) (4)

[REDACTED]

[REDACTED]

[REDACTED]

(b) (4)

(b) (4)

3.2.P DRUG PRODUCT (TM)

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

The AV7909 Anthrax Vaccine (AV7909) DP is a sterile, milky-white suspension intended for IM injection. AV7909 is supplied in a (b) (4) (nominal fill volume: (b) (4) mL; overflow capacity volume: (b) (4) clear borosilicate multi-dose (b) (4) glass vial with a ready-to-sterilize rubber stopper for multi-puncture usage and a flip-top aluminum seal. Each multi-dose vial is filled with approximately (b) (4) of AV7909 DP to ensure a sufficient volume to obtain 10 doses of 0.5 mL per vial. Each 0.5 mL dose of AV7909 DP contains (b) (4) and (b) (4) of adjuvant (CPG 7909).

Overall Reviewer's Assessment of Section 3.2.P.1 (TM):

No deficiencies were identified.

3.2.P.2 Pharmaceutical Development

Excipients:

The compendial excipients and their respective functions in the DP are listed below.

- Aluminum hydroxide (b) (4) is used in the formulation of AVA DS as an adjuvant.
- Sodium chloride is used in the formulation to maintain the (b) (4)
- Formaldehyde solution, (b) (4), is used in the formulation as a preservative by cross-linking with the adsorbed antigens. It also acts as a stabilizer, having the ability to cross link with the adsorbed antigens.
- Benzethonium chloride is a bactericidal cationic quaternary ammonium surfactant used as a topical anti-infective agent.

There are no non-compendial excipients in the DP.

Drug Product Formulation Development:

The AV7909 DP consists of the AV DS formulated with CPG 7909 adjuvant. The AV (b) (4) is (b) (4) in composition to commercial BioThrax. It contains the active ingredient (AV Filtrate, a clarified cell-free sterile filtered culture from a microaerophilic (b) (4) an avirulent non-encapsulated strain of *B. anthracis*), adsorbed onto an $Al(OH)_3$ adjuvant and suspended into a saline solution containing preservatives.

(b) (4)

The DP formulation selected at the end of Phase 1 (0.5 mL (b) (4) CPG 7909 per dose) was unchanged in a Phase 2 clinical study (Lot (b) (4) to determine the dosing schedule. (b) (4) development lots were produced (Lots (b) (4) with the formulation unchanged to confirm the scale-up process from Building (b) (4) to Building (b) (4) pre-PPQ engineering (b) (4), and all PPQ runs (Lots (b) (4) of these PPQ lots (Lots (b) (4) were used in the Phase 3 clinical study.

Physicochemical and Biological Properties:

The physiochemical and biological attributes of the AV7909 DP are (b) (4) BioThrax except for the addition of CPG 7909. CPG 7909 is a synthetic DNA molecule 24 nucleotides in length, with a nuclease-resistant phosphorothioate backbone. CPG 7909 binds Toll-like receptor 9 and induces enhanced antigen-specific antibody responses and natural killer T-cell responses. (b) (4)

Manufacturing Process Development:

(b) (4)


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

Container Closure System:

AV7909 DP is delivered in a (b) (4) (nominal fill volume: (b) (4); overflow capacity volume: (b) (4) clear borosilicate glass multi-dose (b) (4) glass vial with a ready-to-sterilize stopper for multi-puncture usage and 20 mm flip-top aluminum seal.

Emergent demonstrated that the (b) (4) vials were resistant to delamination when subjected to (b) (4) in the presence of AV7909 DP over (b) (4) and when filled with AV7909 DP and stored up to 9 months.

(b) (4)



The manufacturer certifies that the rubber used for the (b) (4) stopper meets the requirements of (b) (4) Elastomeric Closures for Injections. The (b) (4) stopper is acceptable as the AV7909 DP container closure system for commercial use.

Microbiological Attributes:

The AV7909 DP meets the requirements for a sterile dosage form. Two components of the formulation, formaldehyde and benzethonium chloride (b) (4) function as preservatives and prevent microbial growth. The (b) (4) of the preservatives was determined by assessing microbial growth in the absence of these preservatives versus the presence of (b) (4) formaldehyde and either (b) (4) or (b) (4). The combination of formaldehyde and (b) (4) was effective in maintaining (b) (4) of the product at the concentrations present in the DP.

Sterility is confirmed on DP during both release and stability testing.

Aseptic process operations have been validated to demonstrate that the manufacturing process is capable of manufacturing sterile product.

During routine stability, container closure integrity testing (CCIT) and (b) (4) testing is performed to confirm continued sterility of the product.

Compatibility:

AV7909 DP is a sterile, milky-white suspension for IM injection to be administered in a 0.5 mL dose. The DP is not to be reconstituted or diluted prior to administration.

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

A real-time leachables study was conducted on three lots of final drug product (FDP) during stability testing out to 48 months and is described in section 3.2.P.8.1 (Stability). No deficiencies were identified.

3.2.P.3 Manufacture

3.2.P.3.1 Manufacturer(s)

Table 6: Drug Product Manufacturers

Manufacturer	Responsibility
<u>Emergent BioDefense Operations</u> 3500 N. Martin Luther King Junior Blvd. Lansing, MI 48906-9910, USA FEI: 1873886 DUNS: 026489018	<ul style="list-style-type: none"> ▪ Manufacture of AV7909 BDP up to the filling step ▪ In-process control testing ▪ Inspection, packaging, and labeling of DP ▪ Release testing of DP ▪ Disposition of DP ▪ Stability testing of DP (not including Container Closure Integrity Testing, [CCIT], (b) (4) or Sterility)

(b) (4)	<ul style="list-style-type: none"> Stability testing of DP (only CCIT and (b) (4)) Alternative site for testing of Aluminum Content in DP (release and stability)
(b) (4)	<ul style="list-style-type: none"> In-process control testing and release testing (sterility) Alternative site for Sterility testing of DP (stability) Manufacture of DP—filling step only

3.2.P.3.2 Batch Formula

Table 7: AV7909 DP Batch Formula

Component	Quality Standard	Quantity per Batch
Total Adsorbed AV Filtrate	(b)	(4)
Aluminum Hydroxide (b) (4)		
Sodium chloride		
Formaldehyde solution, (b) (4)		
Benzethonium chloride		
WFI		
CPG 7909		
Total Batch Size		

Overall Reviewer's Assessment of Sections 3.2.P.3.1 and 3.2.P.3.2 (TM):




No deficiencies were identified.

3.2.P.3.3 Description of Manufacturing Process

(b) (4)

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

(b) (4)



Overall Reviewer's Assessment of Section 3.2.P.3 (TM):

No deficiencies were identified.

3.2.P.3.4 Controls of Critical Steps and Intermediates

Preparing the (b) (4) CPG 7909 to the target concentration range and confirming the concentration achieved is a critical step. The concentration is determined by measuring

(b) (4) . This method is used as an in-process control during the manufacturing process to quantitatively determine the (b) (4)

(b) (4)

(b) (4)

Overall Reviewer's Assessment of Section 3.2.P.3.4 (TM):

The critical process parameters were identified and are appropriate. The in-process parameters adequately control critical steps, and the in-process testing assures critical criteria are met. No deficiencies were identified.

3.2.P.3.5 Process Validation and/or Evaluation

(b) (4)

Overall Reviewer's Assessment of Section 3.2.P.3.5 (TM):

The (b) (4) PPQ runs encompassed all process stages: production, filling, visual inspection, and DP testing. All in-process parameters, processing times, and hold times were controlled within the specified NORs. All stages of the manufacturing process for all PPQ runs met in-process acceptance criteria, and acceptance criteria for release were met. No deficiencies were identified.

3.2.P.4 Control of Excipients

3.2.P.4.1 Specifications

CPG 7909 adjuvant is a novel excipient added during the AV7909 DP

manufacturing process. Details are included in the CPG 7909 DS review in Section 3.2.S. Specifications for other excipients in the AV7909 DP (benzethonium chloride, (b) (4) formaldehyde solution, sodium chloride and WFI) conform to the (b) (4). The testing of the (b) (4) Aluminum Hydroxide (b) (4) conforms to the current (b) (4) and is also subject to additional in-house supplementary tests for aluminum content, (b) (4) and endotoxin.

Overall Reviewer's Assessment of Section 3.2.P.4.1 (TM):

No deficiencies were identified.

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

(b) (4)

(b) (4)

Overall Reviewer's Assessment of Sections 3.2.P.4.2 and 4.3 (TM):

No deficiencies were identified.

3.2.P.4.4 Justification of Specifications

Emergent's in-house aluminum specification (b) (4) matches the content on the supplier's label, which is in adherence with the (b) (4) requirement of (b) (4).

Emergent's in-house specification for (b) (4) is tighter than the acceptable (b) (4) range specified by the (b) (4) which specifies a (b) (4) range of (b) (4).

The specification for bacterial endotoxins is (b) (4) Endotoxin Units (EU)/mL of Aluminum Hydroxide (b) (4). This limit meets the (b) (4) requirement of (b) (4) of aluminum.

Using the acceptable range of aluminum content for the (b) (4) Aluminum Hydroxide (b) (4) the limit is calculated as (b) (4).

Refer to the DBSQC memo for details regarding the justification of specifications for the following excipients: Benzethonium chloride, formaldehyde solution (b) (4), sodium chloride, and WFI.

Overall Reviewer's Assessment of Section 3.2.P.4.4 (TM):

Refer to the DBSQC memo for details regarding the justification of specifications for the excipients.

3.2.P.4.5 Excipients of Human or Animal Origin

No excipients of human or animal origin are used in the manufacture of the AV7909 Drug Product.

Overall Reviewer's Assessment of Section 3.2.P.4.5 (TM):

Not applicable for this product.

3.2.P.4.6 Novel Excipients

The CPG 7909 adjuvant is a novel excipient used in the manufacture of the AV7909 Drug Product. This novel excipient is reviewed by the adjuvant reviewer.

Overall Reviewer's Assessment of Section 3.2.P.4.6:

Refer to DVP reviewer memo.

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)****Table 8: AV7909 DP Release and Stability Acceptance Criterion**

Test Parameter	Test Method	Acceptance Criterion
Appearance	Visual	Milky-white suspension
Detection of PA (Identity)	(b) (4)	(4)
(b) (4) CPG 7909 (Identity)		
(b) (4) CPG 7909		
Aluminum Content		
Formaldehyde Content		
Sodium Chloride Content		
Benzethonium Chloride (b) (4) content		
Sterility		
Container Closure Integrity		
(b) (4)		
Relative Potency		
(b) (4)		

The specification for AV7909 DP and the acceptance criteria were established based on release and stability data on drug product batches used in development runs, engineering runs, nonclinical studies, clinical studies, and distribution to the Strategic National Stockpile (SNS). The statistical analysis of DP release data included the results from a total of (b) (4) DP lots.

Release acceptance criterion for **Appearance**: Milky-white suspension.

This specification meets the (b) (4) criterion that requires that a qualitative statement describing the physical state, color, and clarity of the DP be provided as part of the specifications.

Release acceptance criterion for **Identity**: Positive by (b) (4) for PA and positive for (b) (4) CPG 7909.

To properly identify the DP against other products produced in the facility (BioThrax), (b) (4) methods are required: Identity by (b) (4) and (b) (4) CPG 7909. The (b) (4) method, used to demonstrate the presence of (b) (4) is qualitative and adequate for the purpose. (b) (4) CPG 7909 is an identity test that is used to specifically confirm the presence of CPG 7909, the active pharmaceutical ingredient found in AV7909, at the time of release. This method is (b) (4)

Release acceptance criterion for (b) (4) **CPG 7909**: (b) (4)
The method used to evaluate aluminum is (b) (4). The acceptance criterion is based on process capability analysis generated from (b) (4) scale engineering lots and is also supported by the (b) (4) CPG 7909 data from the clinical AV7909 DP lots.

Release acceptance criterion for **Aluminum Content**: (b) (4)
The method used to evaluate aluminum is (b) (4). The acceptance criterion is based on process capability analysis of DP. The acceptance criterion is also in accordance with the approved acceptance criterion for BioThrax.

Release acceptance criterion for **Formaldehyde Content**: (b) (4)
The acceptance criteria for formaldehyde in AV7909 DP is based on process capability analysis of DP release data.

Release acceptance criterion for **Sodium Chloride Content**: (b) (4)
The sodium chloride is prepared as an (b) (4) close to the (b) (4) of the body; hence, the acceptance criterion for sodium chloride is based on this and supported by process capability analysis of DP release data.

Release acceptance criterion for **Benzethonium chloride** (b) (4) **Content**: (b) (4)
The acceptance criterion for benzethonium chloride for release is based on process capability analysis of DP release data. Based on clinical experience with both AV7909 and BioThrax, benzethonium chloride content that meets the acceptance criterion has not been found to be toxic to study subjects.

Release acceptance criterion for **Sterility**: Pass.
Sterility testing is performed to ensure that the AV7909 FDP meets the sterility requirements for biological products, (b) (4) Biologics and (b) (4) Sterility. The method and acceptance criterion are compliant with (b) (4) Sterility Testing, (b) (4), and Title 21 Code of Federal Regulations (CFR) Part 610.12 Sterility.

Release acceptance criterion for **Potency**: (b) (4)

The acceptance criterion for potency at release is based on data generated on (b) (4) lots of AV7909 DP manufacture at the (b) (4) scale and is supported by clinical immunogenicity and safety data.

The release acceptance criterion for (b) (4)

The acceptance criterion for (b) (4) is considered an acceptable range as it is effectively in the physiological range and is based on process capability analysis of DP release data.

Overall Reviewer's Assessment of Sections 3.2.P.5.1 and 3.2.P.5.6 (TM):

The acceptance criteria are appropriate to control the critical attributes of the DP and are sufficiently justified. No deficiencies were identified.

3.2.P.5.2 and 3.2.P.5.3 Analytical Procedures and Validation of Analytical Procedures (AV)

In a meeting with DBSQC on 29 June 2022, we agreed that DBPAP would be responsible for review of the (b) (4) method, and that DBSQC and DMPQ would be responsible for review of the other methods. Please refer to the DBSQC and DMPQ reviewers' memos for review of the methods not described below.

AV7909 DP batch testing is performed using several analytical procedures or methods mentioned below and released as per their acceptance criteria. Analytical methods used for AV7909 batch testing are:

- Appearance by Visual Inspection
- Identity by (b) (4)
- Identity of (b) (4) CPG 7909 by (b) (4)
- (b) (4) CPG 7909 content by (b) (4)
- Sterility
- Relative Potency
- Container Closure Integrity Testing (for stability testing only)
- (b) (4) (for stability testing only)

(b) (4)

[REDACTED]

(b) (4)

(b) (4)

(b) (4)

3.2.P.5.5 Characterization of Impurities

There is no evidence of the presence of any impurity (organic/unknown) in the AV7909 FDP.

□ **Overall Reviewer's Assessment of Sections 3.2.P.5.4 and 3.2.P.5.5 (TM):**

No deficiencies were identified.

3.2.P.6 Reference Standards or Materials

Refer to section 3.2.S.5 Reference Standards or Materials.

3.2.P.7 Container Closure System

The AV7909 DP is delivered in a (b) (4) (nominal fill volume: (b) (4); overflow capacity volume: (b) (4) clear borosilicate multi-dose (b) (4) glass vial with a ready-to-sterilize rubber stopper for multi-puncture usage and a 20 mm flip-top aluminum seal. The stopper contains no natural rubber. The vials and stoppers used for AV7909 are standard pharmaceutical container closure systems and are known to be safe and compatible with aqueous products. The suitability of the vials and stoppers was demonstrated as described in section 3.2.P.2.4. Final filled vials of AV7909 DP are packaged with a leaflet in individual cartons made from cardboard, which protect the contents from light and the vials from breakage.

Overall Reviewer's Assessment of Section 3.2.P.5.7 (TM):

No deficiencies were identified.

3.2.P.8 Stability (TM)

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

The proposed shelf life of AV7909 DP is 48 months when stored at 5±3°C. Emergent provided real-time data stability data from (b) (4) DP lots. At the time of final review, 48 months of real time stability data was provided for (b) (4) engineering lots (Lots (b) (4) (b) (4)). Forty-eight (48) months of real time stability data was provided for the (b) (4) PPQ lots used in the phase III clinical trials (Lots (b) (4) (b) (4)). (b) (4) months of stability data is available for one of the (b) (4) engineering lot (b) (4) (b) (4)). Thirty-six (36) months of real-time stability data was provided for an (b) (4) (b) (4) lots. Results for all available time points for the (b) (4) real-time stability lots met acceptance criteria. The proposed shelf-life of 48 months is supported by the stability data.

Leachables testing was performed during stability testing on lots (b) (4) (b) (4) to examine leaching of (b) (4) representative leachable compounds identified in preliminary leachable testing. The (b) (4) (b) (4), were below the LOQ for all time points out to 48 months. Extractables studies identified sodium chloride as the only ion species extracted above the LOQ. An increase in sodium content was not observed at any time point in the DP real-time stability studies.

Table 10: Full-scale AV7909 DP Lots Placed on Stability and Stability Data Available at Time of Review

Lot Number	DOM	Description	Data Provided
(b) (4)	(4)	GMP Engineering Run (b) (4) Phase 2 Clinical Lot	(b) (4)
		GMP Engineering Run (b) (4) Nonclinical Lot	48 months
		GMP PPQ (initial PPQ), Phase 3 Clinical Lot	48 months
		GMP PPQ (initial PPQ), Phase 2 (Drug-Drug Interaction) and Phase 3 Clinical Lot	48 months
		GMP PPQ (initial PPQ), Phase 3 Clinical Lot	48 months
		GMP PPQ (expanded processing and hold times and use of AV Media Blend)	36 months
		GMP PPQ (expanded processing and hold times and use of AV Media Blend)	36 months
		GMP PPQ (expanded processing and hold times and use of AV Media Blend)	36 months
		GMP PPQ (expanded processing and hold times and filling at (b) (4))	36 months
		GMP PPQ (expanded processing and hold times and filling at (b) (4))	36 months
		GMP PPQ (filling at (b) (4))	36 months

The long-term stability study of the (b) (4) lots listed above will be continued to (b) (4) months, and the additional data from these real-time studies will be provided as part of the Annual Report post-licensure.

Stability Tests Performed:

- Appearance by Visual Inspection
- (b) (4) CpG by (b) (4)
- Aluminum Content by (b) (4)
- Formaldehyde Content, (b) (4)
- Benzethonium Chloride (b) (4) Content by (b) (4)
- NaCl Content by (b) (4)
- Sterility, (b) (4)
- Potency, (b) (4)
- (b) (4)
- Container Closure by (b) (4)
- Antimicrobial Effectiveness, (b) (4)

Accelerated Stability Studies.

(b) (4) lots were placed on accelerated stability (Engineering Lot (b) (4) and PPQ Lots (b) (4)). The vials were stored (b) (4). They were (b) (4). All (b) (4) lots met all stability acceptance criteria out to the (b) (4) time point.

Ambient Exposure Challenge.

To support the maximum cumulative amount of time that AV7909 DP could be exposed to ambient temperature during the manufacturing process, filled Lot (b) (4) samples were (b) (4). The (b) (4)-month real-time study is still ongoing, with results passing all acceptance criteria for the stability specification up to 48 months.

In-use Stability Study.

AV7909 is stored in a multi-dose vial, and the healthcare provider can withdraw up to 10 doses (0.5 mL per dose) of the vaccine from the vial using a sterile needle. Between each use of the product, the vial should be stored at $5\pm3^{\circ}\text{C}$, typically in an upright (although sometimes supine) position. A study was performed to support the (b) (4) period proposed for use of the multiple-dose vials. Lot (b) (4), which was less than (b) (4) months from its date of manufacture (b) (4), was used for this study. A lot that is near the end of its shelf life (no less than 36 months of age) will also be tested, and the results will be submitted as a minor amendment to the BLA when available.

This study was performed using the (b) (4) position to simulate the worst-case scenario. At time zero, (b) (4)

This study supports the conclusion that AV7909, when used in accordance with expected typical product usage, demonstrates in-use stability through (b) (4) post-puncture when stored at the recommended conditions ($5\pm3^{\circ}\text{C}$).

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

For each calendar year, one lot of AV7909 will be placed on stability testing post-approval as follows.

At Time 0, 3, 6, 9, 12, 18, 24, 36, and 48 months the following tests will be performed:

- Appearance
- Container Closure
- Potency
- (b) (4)
- Aluminum
- Formaldehyde
- Sodium Chloride
- (b) (4)
- (b) (4) CpG by (b) (4)

(b) (4) and Sterility will be evaluated at 0, 12, 24, 36, and 48 months.

IR#21 (5 January 2023, responses received 10 February 2023, STN 125761/0.35; and 3 April 2023, STN 125761/0.41)

Comment 1:

The stability data you have provided for AV7909 final drug product is not sufficient to support your proposed 48-month shelf life. The stability data you provided in STN 125761/0 only supports a dating period of 36 months. If all the results from the 48-month time point for stability lots (b) (4) are within specification, the dating period can be extended to 48 months pending review of those results. In order to extend your shelf-life claim to 48 months, please submit additional stability data that includes the 48-month timepoint for lots (b) (4)

Emergent's response to Comment 1:

Interim stability data were provided in STN 125761/0.35, extending the available stability data. Additional data were provided in STN 125761/0.41, extending the available data for ongoing stability studies.

Review of response to Comment 1:

The combined data were sufficient to support the requested shelf-life of 48 months for the FDP.

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

The real-time stability studies support the requested shelf life of 48 months for the FDP. The ambient temperature exposure during manufacturing studies supports the acceptance criterion for total cumulative exposure to ambient temperature during manufacturing. The in-use stability study supports in-use stability through (b) (4) post puncture when DP is stored at the recommended conditions (5±3°C). The commitment to place one lot of AV7909 on stability each calendar year and the proposed stability testing are acceptable. No deficiencies were identified.

3.2.A APPENDICES

3.2.A.1 Facilities and Equipment

Reviewed by DMPQ.

3.2.A.2 Adventitious Agents Safety Evaluation

(b) (4)

Control of microbial agents for (b) (4)
AV7909 DP is achieved through strict adherence to current good manufacturing procedures, personnel monitoring, and environmental monitoring of the facilities and equipment. In more than (b) (4) lots to date for AV7909 DP, there have been no sterility failures.

(b) (4)

(b) (4)

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

No deficiencies were identified.

3.2.A.3 Novel Excipients

Not applicable.

3.2.R Regional Information (USA)

Executed Batch Records

Emergent provided a blank master batch record document for the DS and DP and an executed batch record for DS PPQ lot (b) (4) and DP PPQ lots (b) (4).

The information provided is acceptable.

Method Validation Package

Please see section 3.2.P.5.3 for discussion of the method validation package.

Combination Products

Not applicable.

Comparability Protocols

Not applicable.

Other eCTD Modules

Module 1

A. Environmental Assessment or Claim of Categorical Exclusion

Emergent is claiming a Categorical Exclusion from the requirement for an Environmental Assessment for AV7909 based on 21 CFR 25.31(a), as the use of the (b) (4) has not increased versus what has been previously reviewed and approved under the BioThrax BLA. Emergent is also claiming Categorical Exclusion for CPG 7909 under 21 CFR 25.31(c), for substances that occur naturally in the environment, as the approval of the Original BLA will not significantly alter the concentration or distribution of the substance, its metabolites, or degradation products in the environment. No extraordinary circumstances exist that would require an environmental assessment. The action requested qualifies for a Categorical Exclusion and is in accordance with 21 CFR 25.15(d), whereby Emergent has no knowledge that extraordinary circumstances exist.

FDA's agreement with Emergent's proposal claim Categorical Exclusion for AV7909 Drug Product under the cited regulations was communicated to Emergent in the Written Responses to the Pre-BLA CMC Meeting Request (CRMTS #13195) communicated on 27 April 2021, under IND 014451. Upon review of the BLA submission, the finding of no additional environment impact remains appropriate.

B. Reference Product Designation Request

Applicant did not request reference product designation.

C. Labeling Review

Full Prescribing Information (PI):

The package insert was reviewed. The "Dosage Forms and Strengths" section and the "How Supplied/Storage" section were acceptable. Several deficiencies were noted in

the “Description” section and the “Clinical Pharmacology” section. Proposed edits were communicated to Emergent.

Carton and Container Label:

The carton and container labels were reviewed. The CMC information provided was acceptable.

Modules 4 and 5

Module 4

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

Serology assay (TNA) review for nonclinical studies (AV)


The serology assay used to support licensure is a TNA assay, which measures circulating neutralizing antibody levels (*i.e.*, functional immune response) induced by AV7909 DP in clinical and nonclinical studies. My review covers the documents supporting the performance of the TNA assay used for testing serum samples in pivotal nonclinical studies conducted under the AV7909 nonclinical development program and pertinent to the claimed indication of AV7909 DP in this BLA.

Previously, Emergent conducted pivotal efficacy studies in rabbits and NHPs using AVA (BioThrax) vaccine-induced TNA antibodies as the correlate of protection to bridge efficacy responses in animals and humans. Both animal models (rabbits and NHPs) have been successfully used in the nonclinical studies pertinent for the BioThrax PEP indication (STN 103821/5344). However, guinea pigs (instead of rabbits) and NHPs were the two animal models used for nonclinical development of AV7909. Emergent developed and characterized the guinea pig model of inhalational anthrax for use as the small animal model supporting AV7909 development because the activity of CPG 7909 adjuvant present in the AV7909 DP is significantly weaker in rabbits and therefore may not be representative of the human response to CPG 7909. To assess the effectiveness of the proposed AV7909 human dose, immune responses associated with survival in guinea pigs and NHPs were bridged to humans. This bridging relied on the derivation of an immune threshold of protection based on the relationship between immunogenicity and survival in animal efficacy studies, which was bridged to human immunogenicity data to infer clinical benefit. The TNA assay was used as the serology assay to determine this threshold of protection for AV7909. Because the TNA assay is species-independent, it can be used to directly compare functional immune responses across species, thereby providing a mechanism for bridging animal and human immunogenicity data to support licensure of AV7909 under the Animal Rule for this BLA.

For this review, I evaluated the performance of the TNA assay in two pivotal animal studies in guinea pigs (3580-100069467) and NHPs (3655-100072763). Supportive of the guinea pig as an appropriate animal model, several other guinea pig studies were

performed during the IND phase (968-G882407, 969-G882407, and 2940-100027634). Additionally, two animal studies using BioThrax (study 646-N107247 for rabbits and 844-N109502 for NHPs) were used to establish the target protective TNA thresholds for the pivotal Phase 3 clinical study for the AV7909 PEP indication under this BLA. Both studies were reviewed under BioThrax BL (STN 103821/5344) for the PEP indication.

(b) (4)



A high throughput version of the assay (htpTNA assay) was developed and validated for use with rabbits (Protocol #VP2006-138) in 2008 at (b) (4) under the sponsorship of the National Institute of Allergy and Infectious Diseases (NIAID). This version of the assay led to an increase in assay throughput from (b) (4). Although the rabbit htpTNA assay was not used in the nonclinical studies submitted in this BLA, the rabbit htpTNA assay provides a historical basis for the development and validation of htpTNA assays for other animal species. Parameters including precision, dilutional linearity, lower and upper limits of quantitation (LLOQ and ULOQ), limit of detection (LOD), and specificity were validated. The htpTNA validation for NHPs (Protocol #VP2006-152) was also performed at (b) (4). The primary htpTNA validation documents for rabbit and NHP species can be found in NIAID MF (b) (4) in amendments 50 (rabbits) and 59 (NHPs). The TNA assay and associated validations were extensively reviewed under MF (b) (4). The htpTNA assay consistently met the pre-determined acceptance criteria and maintained its performance over time. For the NHP htpTNA assay used in pivotal nonclinical studies, the LLOQ for TNA ED₅₀ and the TNA NF₅₀ (relative to human reference (b) (4)) are (b) (4) respectively. For nonclinical studies, TNA titers less than the LLOQ were assigned the respective LLOQ for statistical analysis.

To support the use of the guinea pig as the animal model for AV7909 nonclinical studies, the htpTNA assay was validated at (b) (4) under Emergent sponsorship (Study VP2016-302) for use with guinea pig serum samples using SOP (b) (4). X-143-09 ("Standard Operating Procedure for the High Throughput Toxin Neutralization Assay (htpTNA) Proper") under IND 014451/075 (reviewed by FDA/CBER reviewers). For the

guinea pig htpTNA assay, all validation criteria were met, and the guinea pig htpTNA assay was deemed appropriate for use in measuring antibody responses to vaccination or infection in the guinea pig model. The LLOQ for TNA ED₅₀ and the TNA NF₅₀ (relative to human standard reference (b) (4)) are (b) (4) respectively. This validated guinea pig htpTNA assay was used as the serology assay to analyze test serum samples from nonclinical studies conducted in support of the AV7909 DP development for the PEP indication.

Importantly, the validated htpTNA assay for NHPs was used for licensure of BioThrax under the Animal rule for the PEP indication (STN BLA 103821/5344). I have no concerns regarding its use in the nonclinical studies to support the indication of AV7909 DP.

Use of htpTNA assay in nonclinical studies in support of AV7909 PEP indication

The approach to establish the TNA level associated with protection against death due to inhalational anthrax for AV7909 was similar to the approach used for BioThrax licensure under the FDA Animal Rule. The two pivotal nonclinical studies, one in guinea pigs (study 3580-100069467) and one in NHPs (study 3655-100072763), were performed to support the licensure of AV7909 DP with the following objectives:

Pivotal Study 3580-100069467 for guinea pigs

The objective of this study was to evaluate the protective efficacy and protective TNA threshold (NF₅₀ and ED₅₀) of AV7909 against a *B. anthracis* spore aerosol challenge on Day 28 or Day 70. The test serum samples from a total of 240 guinea pigs were analyzed using the htpTNA assay at (b) (4).

Pivotal Study 3655-100072763 for NHPs

The goal of this study was to evaluate the protective efficacy and establish a threshold of protection of AV7909 against a *B. anthracis* spore aerosol challenge on Day 28 or Day 70 using the intended human two-dose regimen administered two weeks apart in cynomolgus macaques. The test serum samples from 172 macaques were analyzed using the htpTNA assay at (b) (4).

To ensure that the htpTNA performed consistently over the entire time period for both nonclinical studies, an IR was submitted on October 12, 2021, asking Emergent to provide the versions of the htpTNA assay SOPs used for the nonclinical studies and to include a description of the changes made to the SOP since validation of htpTNA for guinea pigs and NHPs. In response, Emergent provided details regarding the SOPs used for the htpTNA assay for both studies. Test sample analysis for the htpTNA assay for guinea pigs and NHPs was conducted following SOP (b) (4).X-143 versions 8 and 9 “High Throughput Toxin Neutralization Assay (htpTNA) Proper” for nonclinical studies. Specifically, the SOP (b) (4).X-143 version 9 was used for validation of the guinea pig htpTNA assay. I reviewed the information regarding the changes made in SOP

(b) (4).X-143 versions 8 and 9 and concluded that all changes made were minor procedural changes, resulting in no impact to the validation status of the htpTNA assay. Three additional SOPs (SOP (b) (4).X-147 versions 1-3, SOP (b) (4).XV-009, and SOP (b) (4).XV-014 versions 3 and 4) were also used in the analysis, calculation, and reporting of the htpTNA results. All above-referenced SOPs are available via cross-reference to NIAID's MF (b) (4).

A human serum reference standard (b) (4), with an ED₅₀ range of (b) (4), and a positive control (b) (4), with an ED₅₀ range of (b) (4), were used in all pivotal studies using the htpTNA assay, which allowed critical comparisons between assays and studies. Additionally, the same lots of the critical reagents (b) (4) and (b) (4) were used in the two nonclinical studies. Also, to assess the performance and the stability of the htpTNA assay since the time of validation through completion of testing for nonclinical studies, I requested (via an IR sent on October 12, 2021) that Emergent provide the time frame and dates when test serum samples from the nonclinical studies were tested as well as the control charts of ED₅₀ and NF₅₀ values for the reference standard (b) (4) and the positive control (b) (4) used in the htpTNA assay. In response, Emergent submitted a response under IND 014451/233 and provided the descriptive control charts plots for ED₅₀ and NF₅₀ values of (b) (4) from years 2008–2020. The htpTNA assays for both pivotal nonclinical studies were performed in 2017. No aberrant trends were observed for the ED₅₀ or NF₅₀ values for the standard reference serum (b) (4) or the positive control (b) (4) used in the htpTNA assay for all nonclinical studies. The control values appeared to be stable over time, with very few points outside of the standard deviation range. Also, I reviewed the ED₅₀ and NF₅₀ listings of the TNA titers in (b) (4) final reports for the guinea pig and NHP nonclinical studies. The data showed consistent performance of the htpTNA assays, and no aberrant results for TNA titers were observed. Several deviations and event reports (for inadvertent repeats) were noted for both nonclinical studies. However, all of them were minor in action and had no potential to impact to reportable ED₅₀ and NF₅₀ values. The data support the consistent performance of the htpTNA assay performed to collect data for pivotal nonclinical studies.

Overall Reviewer's Assessment for htpTNA Performance for Nonclinical Studies (AV):

I reviewed all the documents relating to htpTNA assay performance. Data supporting assay performance since validation indicate that the htpTNA assays performed consistently. Therefore, the htpTNA assay is deemed suitable for its intended use, and the serologic data generated in the htpTNA assay are valid. No deficiencies were identified. However, IRs were sent to Emergent as described below.

IND IR (12 October 2021, response received 5 November 2021 under IND 14451/233):

Please address the following for the high-throughput (htp)TNA assays used for non-clinical and clinical studies:

Question 1:

- a. For the non-clinical and clinical studies listed in tables 3 & 4 of the briefing package, please identify the amendments containing final validation reports, and any additional amendments that refer to the original validations for the htpTNA assays. If these are not included in your IND 14451, please submit Letters of Cross-reference for the IND or MF for the information.

Emergent's response to question 1a:

Emergent identified the amendments associated with IND 14451 for the final validation reports for our reference. In addition, Emergent asserted that the htpTNA assay used for the AV7909 nonclinical NHP pre-exposure and clinical studies was transferred from the CDC, modified, and validated at (b) (4), under the sponsorship of NIAID, and the original reports are available in NIAID's MF (b) (4). Emergent provided a Letter of Cross-reference to MF (b) (4) in Module 1, Section 1.4 of this BLA submission. Emergent stated that the final validation report for the guinea pig htpTNA assay (VP2016-302) conducted by (b) (4) under Emergent sponsorship was submitted to the IND under Serial No. 0075 and reviewed by CBER/FDA reviewers. This is acceptable, and no further action is required.

- b. Please provide the version of the SOP currently in use for the htpTNA assays for each species used in the non-clinical and clinical studies and any supporting documents that are used in the analyses, calculation, and reporting of results. Please include a description of changes made to the SOP since validation and discuss if the changes have the potential to affect the assay parameters determined during validation or the output of results.

Emergent's response to Question 1b:

Emergent stated that sample analysis for the htpTNA assay for each species was conducted using SOP (b) (4).X-143 "High Throughput Toxin Neutralization Assay (htpTNA) Proper." The versions of the SOP used for each species in the nonclinical and clinical studies were provided with the description of changes made to the SOP since validation. I reviewed the documents, and none of the changes had any potential to affect the assay parameters and performance.

Emergent indicated that three additional supporting documents were also used in the analysis, calculation, and reporting of TNA results. They are as follows:

- SOP (b) (4).X-147 "Analysis of the Toxin Potency Assay (TPA), Screening Assay, and High-Throughput Toxin Neutralization Assay (TNA) Data Using the CDC TNA Software"
- SOP (b) (4).XV-009 "Standard Operating Procedure (SOP) for the operation of the Toxin Neutralization Assay (TNA) Database"

- SOP (b) (4).XV-014 “The Operation of the High-Throughput Toxin Neutralization Assay (htpTNA) Module of the Bioassay Database”

All above-referenced SOPs are available via cross-reference to NIAID’s BB-MF (b) (4)

- c. Please provide control charts of ED₅₀ and NF₅₀ values for the positive control (b) (4) and the reference standard (b) (4) used in the htpTNA assay to assess the performance and the stability of the htpTNA assay since the time of validation through completion of testing samples for Phase 3 study (EBS.AVA.212).

Emergent’s response to Question 1c:

Emergent provided control charts for ED₅₀ and NF₅₀ values of the positive control (b) (4) and the ED₅₀ for reference standard (b) (4) used in the htpTNA assay, starting from January 2007 to completion of testing samples for the Phase 3 study. I reviewed the control charts and did not identify any issues.

- d. Please provide the dates that samples from the pivotal nonclinical and clinical studies were analyzed.

Emergent’s response to Question 1d:

The sample testing dates were provided for the pivotal nonclinical and clinical studies for assessing the performance of the assay.

- e. Please provide a summary of the characterization information for critical reagents used in htpTNA (e.g., reference serum/standards, positive controls, cells, protective antigen, and lethal factor)

Emergent’s response to Question 1e:

A summary of the characterization information available for reference standards, positive controls, and critical reagents used in the htpTNA assay was provided.

Review of responses:

I reviewed all documents provided by Emergent in response to my IR. The responses were adequate to assess the performance of htpTNA used for nonclinical and clinical studies.

Non-clinical Studies (TM)

Overview

Due to the rare occurrence of *B. anthracis* infections and the inability to design ethical studies exposing humans to anthrax, it is not feasible to conduct clinical-endpoint efficacy studies. Therefore, Emergent is pursuing licensure of AV7909 for the PEP indication under the FDA Animal Rule (21 CFR Part 601, Subpart H, "Approval of Biological Products when Human Efficacy Studies are not Ethical or Feasible").

Passive transfer animal studies were performed with purified human immune globulin generated by vaccination with BioThrax in support of the PEP licensure. These studies confirmed the ability of neutralizing anti-PA antibodies to protect animals against death from anthrax disease. Because AV7909 is composed of the AVA bulk DS, which is made with the (b) (4) ingredients at the (b) (4) concentrations and is manufactured using the (b) (4) process as BioThrax, it shares the major antigenic component (PA) with BioThrax. Therefore, the protective effect of AV7909-induced neutralizing anti-PA antibodies is not expected to be different from that of the BioThrax-induced antibodies, and the protective titer required to achieve protection of subjects is reasonably likely to be comparable to that of BioThrax.

PA-based vaccines, such as AV7909, confer protection by eliciting anthrax LT-neutralizing antibodies. The ability of PA-based vaccines to generate a robust, protective TNA response has been demonstrated in multiple animal models including guinea pigs, rabbits, and NHPs. The pathogenic mechanisms resulting in inhalational anthrax are well characterized and shown to closely resemble the human disease in both the rabbit and NHP (*cynomolgus macaque*) aerosol challenge models. These models served as the pivotal animal models supporting BioThrax PEP licensure. Because the activity of the CPG 7909 adjuvant has been observed to be significantly weaker in rabbits than in humans, the rabbit does not provide an appropriate model for the evaluation of CPG 7909-adjuvanted vaccines. To provide a suitable second animal to demonstrate the effectiveness of AV7909, Emergent developed a guinea pig model of inhalation anthrax. A natural history study of inhalation anthrax in guinea pigs demonstrated that the course of inhalation anthrax disease and the resulting pathology in guinea pigs were comparable to those seen in rabbits and NHPs.

In the pre-exposure prophylaxis animal models, animals were vaccinated, and the TNA response was measured prior to challenge. The relationship between pre-challenge TNA levels and survival post-challenge was evaluated. The pre-exposure studies with AV7909 in guinea pigs and NHPs demonstrated that the vaccine-induced immune response protected animals against death from anthrax in a dose-dependent manner.

In the post-exposure animal model, animals were first challenged with a lethal dose of anthrax spores, then vaccinated in combination with antibiotics. This study demonstrated the ability of the vaccine to increase survival after the cessation of antibiotic treatment, as compared with antibiotic treatment alone. In addition to qualifying the animal model, these studies demonstrated that the vaccine was reasonably likely to confer benefit and justified clinical development.

To assess the effectiveness of the proposed AV7909 human dose, neutralizing antibody responses associated with survival in animals were bridged to human immunogenicity data to infer clinical benefit. This approach was discussed at the VRPBAC meeting held on November 16, 2010, and was considered by the committee to be appropriate for demonstration of anthrax vaccine efficacy. The TNA assay was used to determine this threshold of protection for AV7909. The approach to establish the TNA level associated with protection against death due to inhalational anthrax for AV7909 is similar to that used to license BioThrax under the FDA Animal Rule. Six pre-exposure prophylaxis studies in guinea pigs and NHPs (three studies in each species) were conducted. Guinea pigs and NHPs were vaccinated on Days 0 and 28 or on Days 0 and 14. Vaccination on days 0 and 14 mimics the intended human dosing schedule for PEP. Animals were subsequently challenged via inhalation with aerosolized *B. anthracis* Ames spores at the target dose level exceeding the LD₅₀ by 200-fold (*i.e.*, 200 LD₅₀) on Day 28 or Day 70. Pre-challenge TNA levels were assessed, and the thresholds of TNA levels associated with 70% probability of survival were calculated based on logistic regression analyses. The AV7909 TNA threshold of protection was comparable between the two animal models and between the two immunization schedules. Furthermore, the Day 28 TNA threshold of protection obtained with the Day 0, 14 schedule was similar to the Day 69/70 threshold of protection obtained with the Day 0, 14 or Day 0, 28 immunization schedules.

The FDA advised that the most conservative target protective TNA NF₅₀ threshold of 0.56, derived from the pivotal BioThrax rabbit PEP study (Study 646-N107247), be used as the threshold for the first of two co-primary clinical immunogenicity endpoints: Number of subjects achieving a TNA NF₅₀ value ≥ 0.56 at day 64 in the pivotal AV7909 Phase 3 study (Study EBS.AVA.212).

Development of the Guinea Pig Model of Inhalation Anthrax

Emergent developed the inhalation anthrax (b) (4) guinea pig model in a series of studies. A study was conducted to determine the median inhalation lethal dose of *B. anthracis* (b) (4) spores in guinea pigs. Subsequent studies characterized the natural history of inhalation anthrax in guinea pigs, which demonstrated that the course of disease and the resulting pathology in guinea pigs are similar to those seen in rabbits and NHPs, as well as in humans. Subsequent studies were conducted to evaluate the tolerability and pharmacokinetics of ciprofloxacin in guinea pigs and to develop a partially curative antibacterial regimen in the guinea pig model. These studies enabled studies demonstrating the added survival benefit of both the antibiotic treatment and vaccination compared to the administration of antibiotic treatment alone.

Rationales for Pre- and Post-Exposure Studies:

Following a large anthrax exposure, a small percentage of spores can remain dormant in the lungs for an extended period of time. These residual spores can germinate and establish disease more than sixty days after the initial exposure. If use of AV7909 is required in a post-exposure situation, the purpose of the vaccine is to protect against residual spores that germinate after cessation of antibiotic use. In the post-exposure scenario for anthrax vaccines, antibiotics are administered for 60 days. The vaccine is

administered concurrently or very shortly after initiation of antibiotic therapy. Thus, the vaccine is administered with sufficient time for induction of a protective immune response before germination of residual spores remaining after cessation of antibiotics would occur. The pivotal pre-exposure prophylaxis animal studies were designed to demonstrate vaccine-induced protection and to identify the TNA titers required to confer protection from exposure in such a scenario. This approach was discussed and approved by VRBPAC during the meeting of November 16, 2010.

Post-exposure prophylaxis animal studies were designed to demonstrate an added benefit of vaccination in combination with antibiotic treatment over antibiotic treatment alone following an anthrax spore exposure.

Pre-Exposure Prophylaxis Studies:

In two guinea pig studies (968-G882407 and 2940-100027634) and two NHP studies (970-G882407 and 3124-100043225), groups of animals were immunized on Days 0 and 28 with dilutions of AV7909, a single dose level of AVA, or placebo (adjuvant alone or sterile saline). The animals were challenged on Day 70 by inhalation with 200 LD₅₀ of aerosolized *B. anthracis* spores. A 70% probability of survival was associated with Day 69 TNA NF₅₀ titers ranging from 0.063–0.081 in the guinea pig studies and Day 70 NF₅₀ titers of ranging from 0.107–0.262 in the NHP studies.

Emergent conducted two additional studies (one in guinea pigs [3580-100069467] and one in NHPs [3655-100072763]), intended to more closely mimic the intended clinical regimen of two vaccinations two weeks apart. Animals were immunized on Days 0 and 14 and challenged on day 28 (to determine whether protective TNA levels could be achieved at an earlier timepoint) or on Day 70. Pre-challenge TNA NF₅₀ titers of 0.072 for guinea pigs and 0.151 for NHPs were associated with a 70% probability of survival following challenge on Day 28. Pre-challenge TNA NF₅₀ titers of 0.081 and 0.262 in guinea pigs and NHPs, respectively, were associated with a 70% probability of survival following challenge on Day 70.

In BioThrax Studies 646-N107247 and 844-N109502, groups of rabbits or NHPs, respectively, were immunized on Days 0 and 28 with dilutions of BioThrax or placebo (adjuvant alone or phosphate-buffered saline) and subsequently challenged on Day 70 with 200 LD₅₀ of aerosolized *B. anthracis* spores. A pre-exposure TNA NF₅₀ level of 0.56 corresponded to a 70% probability of survival in rabbits, and a pre-exposure TNA NF₅₀ level of 0.29 corresponded to a 70% probability of survival in NHPs.

Five additional rabbit pre-exposure prophylaxis studies were performed using BioThrax (studies 2396-100009122, 2453-100009155, 2454-100009115, 2455-100009155, and 3237-100020326). These studies showed that TNA NF₅₀ thresholds in the range of 0.19–0.29 correlated with 70% rabbit survival. Logistic regression analysis of pooled study data from these AVA-immunized rabbits (n=632) showed a TNA NF₅₀ threshold of 0.24 was associated with a 70% probability of survival. The NF₅₀ value of 0.240 obtained with the pooled rabbit data analysis is consistent with the NF₅₀ value of 0.294 obtained in AVA NHP Study 844 (the pivotal NHP study for licensure of BioThrax for the

PEP indication). The results of the five additional rabbit studies suggest that the original rabbit study yielding the 0.564 NF₅₀ threshold overestimated the TNA threshold level, likely due to several rabbits that had high TNA titers (NF₅₀ > 0.6) and died of anthrax. An additional factor may be that there were few surviving or non-surviving animals with TNA titers around the 0.24 NF₅₀ TNA threshold in the original study.

Therefore, Emergent proposed using the AVA-immunized NHP TNA threshold of protection NF₅₀ level from NHP study 844 (0.294 NF₅₀), demonstrating non-inferiority of the TNA titers induced by AV7909 relative to BioThrax at Day 64, as the second co-primary endpoint, to which CBER agreed.

Table 11: Pre-Exposure Prophylaxis Studies Using AV7909

Study Number	Species	Vaccination Schedule	Challenge Day	NF ₅₀ Associated with 70% Survival
968-G882407	Guinea Pig	Day 0 and 28	Day 70	0.064
2940-100027634	Guinea Pig	Day 0 and 28	Day 70	≤LLOQ
970-G882407	NHP	Day 0 and 28	Day 70	0.154
3124-100043225	NHP	Day 0 and 28	Day 70	0.210
3580-100069467	Guinea Pig	Day 0 and 14	Day 28	0.072
3580-100069467	Guinea Pig	Day 0 and 14	Day 70	0.081
3655-100072763	NHP	Day 0 and 14	Day 28	0.151
3655-100072763	NHP	Day 0 and 14	Day 70	0.262

Table 12: Pre-Exposure Prophylaxis Studies Using BioThrax

Study Number	Species	Vaccination Schedule	Challenge Day	NF ₅₀ Associated with 70% Survival
646-N107247	Rabbit	Day 0 and 28	Day 70	0.56
844-N109502	NHP	Day 0 and 28	Day 70	0.29
2396-100009122	Rabbit	Day 0 and 28	Day 70	0.21
2453-100009115	Rabbit	Day 0 and 28	Day 70	0.29
2454-100009115	Rabbit	Day 0 and 28	Day 70	0.22
2455-100009115	Rabbit	Day 0 and 28	Day 70	0.19
3237-100020326	Rabbit	Day 0 and 28	Day 70	0.20

The non-clinical animal studies demonstrated that AV7909 induced a rapid TNA response that protected a large proportion of animals from death due to inhalation

anthrax in a dose-dependent manner. The TNA thresholds of protection were similar between the two animal models and were not impacted by vaccination schedule or challenge time point. These studies provide the pivotal animal data for AV7909 PEP licensure and support the TNF NF₅₀ thresholds selected to estimate protection in the clinical trials. The co-primary clinical study immunogenicity endpoints for the pivotal phase 3 trial EBS.AVA.212, which used these animal-derived threshold values, were:

Primary Study Endpoints—Immunogenicity:

1. Lower bound of the two-sided 95% confidence interval (CI) is $\geq 40\%$ for the proportion of AV7909 subjects in Groups 1–3 (three lots pooled) achieving a TNA NF₅₀ ≥ 0.56 on Day 64. For this endpoint, the threshold of protection (0.56) is based on the Day 70 TNA NF₅₀ value associated with 70% survival in rabbits administered BioThrax on Days 0 and 28 and challenged on Day 70 with *B. anthracis* spores (Study 646). This endpoint applies the most conservative threshold associated with 70% protection in any of the non-clinical animal model studies and therefore is acceptable.
2. At Day 64, non-inferiority of AV7909 to BioThrax at Day 64 as determined by the one-sided lower 95% CI of the difference in the proportion of AV7909 subjects (three lots pooled) with a TNA NF₅₀ ≥ 0.29 and the proportion of BioThrax subjects with a TNA NF₅₀ value ≥ 0.29 being greater than -15%. For this endpoint, the threshold of protection of 0.29 is based on the Day 70 TNA NF₅₀ value associated with 70% survival in NHPs administered BioThrax on Days 0 and 28 and challenged on Day 70 (Study 844). This NF₅₀ threshold is further supported by the pooled rabbit study data that demonstrated a comparable NF₅₀ value threshold of 0.240 associated with 70% protection.

Post-Exposure Prophylaxis Study:

To establish proof-of-concept of AV7909 in a PEP regimen, Study 969-G882407 was conducted to evaluate the ability of post-exposure vaccination with AV7909 to increase animal survival compared to that observed with post-exposure antibiotic treatment alone.

- Guinea pigs were challenged via the inhalation route with 200 LD₅₀ of aerosolized *B. anthracis* (b) (4) spores on Day 0.
- Animals were treated with 7.5 mg/kg of ciprofloxacin three times daily for two weeks (Days 1-14).
- Animals were vaccinated by IM on Days 1 and 8 with dilutions of AV7909 or a single dose level of AVA.
- Ciprofloxacin-only and saline-only control groups were also included.

Mortality data demonstrated that AV7909, administered in combination with ciprofloxacin, protected guinea pigs from death due to anthrax in a dose-dependent manner and provided a significant added survival benefit compared to the ciprofloxacin treatment alone. This study demonstrated the added benefit of co-administration of AV7909 with antibiotic relative to antibiotic treatment alone.

Overall Reviewer's Assessment of Nonclinical Studies (TM):

Pivotal animal studies demonstrated that AV7909 induced a rapid TNA response that protected a large proportion of animals from death due to inhalation anthrax in a dose-dependent manner. The TNA thresholds of protection were similar between the animal models and were not impacted by vaccination schedule or challenge time point. These studies provided the animal data that support AV7909 PEP licensure and support the TNF NF₅₀ thresholds selected to estimate protection in the clinical trials. No deficiencies were identified. Additional non-clinical animal studies provided proof of concept that AV7909 given post exposure with antibiotic therapy provides added benefit over antibiotics alone.

Module 5**Serology (TNA) assay review for clinical studies (AV)**

This review memo covers my review of the documents supporting the performance of the TNA assay used for quantitating neutralizing antibodies in serum samples from the following clinical studies: Phase 1 (EBS.AVA.201), Phase 2 (EBS.AVA.208 and EBS.AVA.210), and the pivotal Phase 3 study (EBS.AVA.212). The focus of my review is to assess the performance of the serology TNA assay used for the pivotal Phase 3 study.

To assess the performance of the htpTNA assay to quantitate responses to AV7909 for the PEP indication, I reviewed multiple sections of the BLA submission as well as amendments in response to my IRs.

Serologic Assay for Anthrax PA

A pan-species htpTNA assay was originally validated for use with rabbits in 2008 and submitted to NIAID's MF (b) (4). Validation parameters included precision, dilutional linearity, LLOQ and ULOQ, limit of detection (LOD), specificity, and plate location effects. Later, the htpTNA assay was validated for use with human serum samples using human reference serum (b) (4), with the following results:

- LOD for the ED₅₀ is (b) (4)
- LLOQ for the ED₅₀ is (b) (4)
- LOD for the NF₅₀ is (b) (4)
- LLOQ for the NF₅₀ is (b) (4)

For immunogenicity calculations, TNA NF₅₀ values that were below the LLOQ were reported as 0.032, which is half the LLOQ of the assay. All validation reports, SOPs, and related documents for the human htpTNA are cross referenced to NIAID MF (b) (4). The htpTNA assay for human serum samples consistently meets the pre-determined specifications and maintains the same level of performance over time. Importantly, the same htpTNA assay was used for licensure of BioThrax under the Animal Rule for the PEP indication (STN BLA 103821/5344).

Clinical samples were tested using SOP (b) (4).X-143 (versions 9-11) “High Throughput Toxin Neutralization Assay (htpTNA) Proper.” To ensure that the htpTNA performs consistently over the time period from validation through testing of clinical samples, an IR was submitted to the sponsor on October 12, 2021, asking Emergent to provide a list of the changes incorporated in the different versions of SOP (b) (4).X-143 since the validation and to state whether the changes have the potential to affect the assay performance or the output of results. In response, Emergent provided (under IND 014451/233) a summary of changes made since validation. I reviewed the information provided by the sponsor and concluded that all changes made in different versions of SOP were minor procedural changes, and these changes were not substantive enough to impact the validation status. However, one change incorporated in version 11 (used in Phase 2, EBS.AVA.210 and pivotal Phase 3, EBS.AVA.212 studies) was the expansion of serum stability duration (*i.e.*, (b) (4)). Upon review, I concluded that serum stability duration change has no impact on the validation and performance of the assay. Three additional SOPs (SOP (b) (4).X-147 versions 1-3, SOP (b) (4).XV-009 and SOP (b) (4).XV-014 versions 3 and 4) were also used in the analysis, calculation, and reporting of the htpTNA results. All above-referenced SOPs are available via cross-reference to NIAID’s MF (b) (4).

A human serum reference standard, (b) (4), with an ED₅₀ range of (b) (4), and a positive control (b) (4), with an ED₅₀ range of (b) (4), were used in all clinical studies except for a Phase 1 study (EBS.AVA.201), where (b) (4) with a ED₅₀ range of (b) (4) was used as a positive control. Additionally, the same lots of the critical reagents rPA ((b) (4); lot (b) (4)) and rLF (b) (4); lot (b) (4) were used in each of the clinical studies. Testing of serum samples using the htpTNA was performed between 2011 and 2020. In order to ensure that the htpTNA performs consistently over time from validation until the completion of clinical studies, I asked Emergent (via the IR submitted on October 12, 2021) to provide the time frame and dates when serum samples from the clinical studies were tested as well as the control charts of ED₅₀ and NF₅₀ values for the reference standard (b) (4) and the positive controls (b) (4) used in the htpTNA assay, trending graphs, and descriptive statistics for monitoring assay performance. Emergent submitted the information under IND 014451/233 and provided the descriptive control charts plots for ED₅₀ and NF₅₀ values of (b) (4) and (b) (4) from years 2008–2020. Upon review, no aberrant trends were observed for ED₅₀ or NF₅₀ values for the controls, with very few out of range. The quality control values appear stable over time, with very few points outside of the respective standard deviation range. The data support the consistent performance of the htpTNA assay since validation.

Review of the performance of htpTNA assay used in clinical studies

Here, I include a summary of the performance of the htpTNA in Phase 1 and 2 studies, as these clinical studies were reviewed under IND 014451. In addition, I am providing a complete review of htpTNA assay performance for the pivotal Phase 3 study.

Phase 1 clinical study (EBS.AVA.201)

In this study, the TNA assay was used to evaluate the immunogenicity of different formulations of AV7909. Testing of serum samples was performed using SOP (b) (4).X-143 v. 5 for the htpTNA assay at (b) (4) in 2011. The reportable values for each study sample were the median of at least two independent results for the ED₅₀ and NF₅₀. I reviewed the data submitted by Emergent in response to our IR (submitted on October 12, 2021, and described in depth in section 4 of this review memo) for the controls (b) (4) from validation through completion of testing of serum samples for this Phase 1 study. No aberrant trend was observed for ED₅₀ or NF₅₀ values for the controls during the Phase 1 study testing of serum samples. The data support the consistent performance of the htpTNA assay during the Phase 1 study.

Phase 2 clinical study (EBS.AVA.208)

Primary objectives of this study were to assess the safety and immunogenicity as measured by the TNA NF₅₀ values for each study subject at Day 63. Testing of serum samples was performed using the validated htpTNA assay at (b) (4) in 2014. The SOP (b) (4).X-143 (v. 6 and 7) was used to perform the htpTNA. For the seroconversion calculation, TNA NF₅₀ values that were below the LLOQ were replaced with 0.064 to calculate the (b) (4) rise. Control charts for ED₅₀ and NF₅₀ values for (b) (4) during the testing period showed no aberrant trends. The data support the consistent performance of the assay during the Phase 2 clinical study.

Phase 2 clinical study (EBS.AVA.210)

For this study, the htpTNA assay was used to evaluate the NF₅₀ on Day 37 (two weeks after the final dose of AV7909) following two IM doses of AV7909 with and without concurrent oral administration of ciprofloxacin or doxycycline. Testing of serum samples was performed using the validated htpTNA assay at (b) (4) in 2020. The SOP (b) (4).X-143 (v. 11) was used to perform the htpTNA. For this study, a total of 379 test serum samples were tested. Some test samples were repeat tested, and the final reportable value was the median of all individual passing ED₅₀ and NF₅₀ values (up to four values). The data (b) (4) study number B0510) were reviewed to confirm that the htpTNA assays performed adequately and that no data were inappropriately excluded from analysis. No inconsistencies or unusual number of samples requiring additional testing were noted. Several deviations were noted, but most of them were minor technical errors with no potential impact on reportable ED₅₀ and NF₅₀ values. ED₅₀ and NF₅₀ values for the controls during the testing period showed no aberrant trend. The data support the consistent performance of the assay during this Phase 2 clinical study.

Pivotal Phase 3 clinical study (EBS.AVA.212)

The objective of this study was to evaluate AV7909 lot-to-lot consistency, immunogenicity, and safety for PEP of anthrax in healthy adults.

Testing of serum samples from the pivotal Phase 3 clinical study using the htpTNA was performed using the SOP (b) (4).X-143 v.11 at (b) (4). A total of 10,943 human serum samples were tested during 2019 and 2020. There were (b) (4) plates run for the entire study with an invalidity rate of less than 8% (b) (4) plates failed to meet acceptance criteria). The average ED₅₀ values for (b) (4) were well within the acceptable ranges of (b) (4) respectively. An IR was submitted on September 14, 2022, asking Emergent to provide a summary of the invalid plates including the plate ID, date, and reasons for the invalidity, to gain insight into the performance of the assay. In response, the sponsor provided (STN 125761/0.20) a summary of all invalid plates with reasons for the invalidity. Upon review of the data provided, no specific reason/trend or a time-period was observed for invalidity of plates. This assures that htpTNA functioned appropriately for the testing of serum samples from the pivotal Phase 3 study.

Additionally, I requested (IR submitted on July12, 2022) the criteria used for the retesting and /or replacement of specific data points in this study. In response (STN 125761/0.8), Emergent stated that no test serum samples were retested. There were two occasions when two aliquots from the same timepoint (Day 1, sample collected pre-vaccination) for two subjects were received at (b) (4) and tested. There was no impact on analysis, because in both instances, the reported NF₅₀ values were below the htpTNA assay LLOQ and 0.032 value (which was half of the LLOQ of the assay). The htpTNA ED₅₀ and NF₅₀ data for all the serum samples analyzed in the pivotal Phase 3 clinical study were reviewed (provided in (b) (4) study number B05158) to confirm that the htpTNA assay performed adequately and that no data were inappropriately excluded from analysis. No inconsistencies or unusual number of samples requiring additional testing were noted. Several deviations were reported and reviewed; most of them were minor in action and had no potential to impact to reportable ED₅₀ and NF₅₀ values. An event report describing a deviation (DR-B05158(b) (4)-0014) was noted i multiple (n=41) test serum samples were retested inadvertently due to a technical error (incorrectly assigned pass/fail initially). The test procedure, SOP (b) (4).X-143 v.11, has a provision to appropriately handle the inadvertent repeats. I have no concerns regarding these inadvertent repeats.

Overall Reviewer's Assessment for htpTNA Performance for Clinical Studies (AV):
--

I reviewed all the documents relating to the htpTNA assay performance for the clinical studies. The data demonstrate adequate performance of the htpTNA as a serology assay to analyze TNA titers for the pivotal Phase 3 clinical study to support AV7909 licensure. Therefore, the htpTNA assay is deemed suitable for its intended use, and the serologic data generated in the htpTNA assay for the clinical studies are valid.

Relevant IRs:

IR#5 (12 July 2022, responses received 26 July 2022, STN 125761/0.8; and 17 August 2022, STN 125761/0.11):

For the high throughput Toxin Neutralizing Antibody assay (htpTNA) assay used in clinical studies:

Comment 6a:

Please provide a summary of the critical reagents used in the htpTNA. Please use the same format as Table 2 (TNA Assay Critical Reagent Tracking Data for Non-Clinical Studies) in Section 2.6.4 (Pharmacokinetic Summary).

Emergent's response to Comment 6a:

Emergent provided a summary of critical reagents used in the htpTNA, as well as the time frames for htpTNA samples testing from clinical Studies EBS.AVA.201, EBS.AVA.208, EBS.AVA.210, and EBS.AVA.212. The corresponding versions of htpTNA SOP (b) (4).X-143 in use during the testing and tracking data for the reference standard (b) (4) and the quality control samples were also provided.

Review of response to Comment 6a:

This is acceptable. However, on 17 August 2022, Emergent submitted another amendment (STN125761/0.11) related to our Comment 6a to correct the critical reagent tracking data information provided for Study EBS.AVA.201. Emergent noticed a typographical error for percent coefficient of variation (from 12.1 % to 21.1%) for NR-717.

Review of additional response to Comment 6a:

This is acceptable.

Comment 6b:

If you retested samples in your htpTNA and replaced specific data points in studies EBS.AVA.212 and EBS.AVA.210, please provide a summary of retesting either as part of the Clinical Study Report or separately. In this summary, we request you include a listing of the values replaced during data cleaning, reasons for sample retesting, and an assessment of the impact of the retesting and replacement of values.

Emergent's response to Comment 6b:

Emergent asserted that no sample retesting was performed in clinical studies EBS.AVA.210 and EBS.AVA.212, and no originally reported final values for the test samples were replaced.

Review of response to Comment 6b:

This is acceptable.

IR# 9 (14 September 2022, response received 28 September 2022, STN125761/0.20)

Comment 2:

In response to our July 12, 2022, Information Request #5, comment #6, you provided the summary of the critical reagents used in the htpTNA for different clinical studies in table 1(Section 1.11.3 Clinical Information Amendment). We note that for the study (EBS.AVA.212), (b) (4) plates were valid out of a total of (b) (4) plates tested for htpTNA. Please provide in tabular format a summary of the invalid plates including the plate ID, date, and reason(s) for the invalidity.

Emergent's response to Comment 2:

Emergent provided a tabular summary including the plate ID, date, and reason(s) for the invalidity of each plate tested for htpTNA. There were (b) (4) failed plates.

Review of response to Comment 2:

There was no trend or reason for the failure to meet the acceptance criteria. This is acceptable.