



## DBSQC/OCBQ ANALYTICAL METHOD REVIEW MEMO

**To:** The file STN 125731/0

**From:**

Reviewer	Role	Date Finalized	Stamp	Supervisor	Stamp
Kouassi Ayikoe, Ph.D.	Lead Reviewer	03/16/2021		Tao Pan, Ph.D. /Kori Francis	
Ritu Agarwal, Ph.D.	Reviewer	4/12/2021		Tao Pan, Ph.D. /Kori Francis	
Hyesuk Kong, Ph.D.	Reviewer	4/01/2021		Kenny James, D.Sc.	
Anil Choudhary, Ph.D., MBA.	Reviewer	03/31/2021		Muhammad Shahabuddin, Ph.D.	

**Through:** Maryna Eichelberger Ph.D., Division Director, DBSQC/OCBQ/CBER/FDA

**Applicant:** Pfizer Inc.

**Subject:** Analytical method Review Memo for BLA 125731/0

---

**Recommendation:** Approval

### Summary of Reviews and Conclusion:

On September 03 and October 10, 2020, Pfizer submitted Part 1 and Part 2 of their rolling Biologics License Application submission (BLA STN 125731/0) for 20-valent Pneumococcal Conjugate Vaccine (20vPnC).

This document contains DBSQC reviews of the analytical methods and their validations as well as the responses to all information requests. All methods reviewed in this memo were described and validated adequately and are suitable for lot-release testing of the product. The following analytical methods used for lot release testing of 20vPnC were reviewed.

1. (b) (4)  
(Kouassi Ayikoe)
2. (b) (4) (Kouassi Ayikoe)

3. (b) (4) (Kouassi Ayikoe)
4. Aluminum Concentration in 20vPnC Drug Product (DP) (Kouassi Ayikoe)
5. Total Protein (b) (4) in 20vPnC DP (Kouassi Ayikoe)
6. Appearance for (b) (4) 20vPnC drug product (Ritu Agarwal)
7. Determination of (b) (4) 20vPnC drug product (Ritu Agarwal)
8. Assay for (b) (4) (Ritu Agarwal)
9. Quantitation of Polysorbate 80 by (b) (4) for 20vPnC DP (Ritu Agarwal)
10. Volume of injection for 20vPnC DP (Ritu Agarwal)
11. Sterility Test Qualifications for 20vPnC (b) (4) DP (Hyesuk Kong)
12. (b) (4) (Hyesuk Kong)
13. Bacterial Endotoxin Test Method for (b) (4) DP (Hyesuk Kong)
14. Review of Validation of Identity (ID) Assay for (b) (4) DP (Anil Choudhary)

## Documents Reviewed

-125731/0.1, Amendment Received 10/08/2020 – Seq. 0002; DATS# 925511

- 1.1.2 FDA Forms 356H – 10/08/2020
- 1.2 Cover Letters –Information for Reviewers – Rolling submission 2; Final of Original Submission – 10/08/2020.
- 2 Common Technical Document Summaries: QOS.
  - 2.3.S. QOS – Control of Drug Substances – Serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 33F.
  - 2.3.S. QOS – Control of Drug Substance – CRM<sub>197</sub>-CY
  - 2.3.S. QOS – Control of Drug Substance – CRM<sub>197</sub>-DM
  - 2.3.P. QOS – Control of Drug Product – 20vPnC
- 3.2.S.4. Control of Drug Substance: CRM<sub>197</sub> and Serotypes (1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 33F).
  - 3.2.S.4.1 Specifications
  - 3.2.S.4.2 Analytical Procedures
  - 3.2.S.4.3 Validation of Analytical Procedures
  - 3.2.S.4.5 Justification of Specifications
- 3.2.P.5. Control of Drug Product
  - 3.2.P.5.1 Specifications

- 3.2.P.5.2 Analytical Procedures
- 3.2.P.5.3 Validation of Analytical Procedures
- 3.2.P.5.6 Justification of Specifications

-125731/0.18 - 1.11.1 Quality Information Amendment; Response to IR dated 16 Feb. 2021

-125731/0/10 and 125731/0/25 – Amendments received 25 January and 19 March 2021.

-125731/0: M3 CMC, Quality

-INDs and BLAs: Master File IND 17039

- Document Number: SOP- 13496 (for 20V)- (b) (4) assay for identification of (b) (4), polysaccharides and proteins in vaccine material (b) (4).
- Document Number SOP- 32763- (STM-I-1006 for newly introduced 7 serotypes), (b) (4) assay for identification of polysaccharides and proteins conjugates.
- Document Number: SOP- LAB-4663 (TMS- 000000057 for 13V)- (b) (4) assay for identification of (b) (4), polysaccharides and CRM<sub>197</sub> in vaccine materials (b) (4).
- Validation Reports for Analytical Procedures- Identity (Saccharide/CRM<sub>197</sub>) Section 3.2.S.4.3 and Section 3.2.P.5.3.

## Background

Pfizer has developed a 20-valent Pneumococcal Conjugate vaccine (20vPnC) with expanded serotype coverage for prevention of pneumococcal disease beyond that of currently licensed Prevnar13 (13vPnC) in adult and pediatric populations. The vaccine is modeled after Prevnar 7 and Prevnar 13 and contains capsular polysaccharides of *Streptococcus pneumoniae* serotypes covalently linked to CRM<sub>197</sub> protein (a nontoxic variant of diphtheria toxin). The vaccine contains capsular polysaccharide conjugates for the serotypes present in Prevnar 13 (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F) and seven additional serotypes (8, 10A, 11A, 12F, 15B, 22F and 33F).

The 20vPnC contains the capsular polysaccharides of *Streptococcus pneumoniae* serotypes, each covalently linked to the cross-reactive material 197 (CRM<sub>197</sub>) protein (a nontoxic variant of diphtheria toxin). The 20vPnC vaccine contains the same capsular polysaccharide conjugates for the serotypes as present in Prevnar 13 (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F) and seven additional serotypes (8, 10A, 11A, 12F, 15B, 22F and 33F). The 20vPnC is available as a suspension for intramuscular injection in 0.5 mL single-dose prefilled syringes. The 20vPnC DP is a sterile liquid suspension for intramuscular injection, supplied in a 0.5 mL single-dose

pre-filled syringe. Each dose contains 20 monovalent bulk conjugates (MBC) in a (b) (4) succinate buffer containing (b) (4) sodium chloride and (b) (4) polysorbate 80, at (b) (4); with aluminum phosphate at (b) (4) as an adjuvant.

The 20 MBC are formulated into the 20vPnC DP and final product release tested at the Pfizer (b) (4). Prevnar 13 (i.e., 13vPnC) is manufactured at Wyeth Pharmaceuticals in (b) (4). The seven new pneumococcal polysaccharide DS and CRM<sub>197</sub> are manufactured at the Pfizer (b) (4) facility in (b) (4). The Prevnar 13 manufacturing processes were reviewed and licensed by the FDA in BLA 125324. Pfizer re-evaluated the approved analytical procedures for the testing of Prevnar 13 and verified they were appropriate for their intended use as a DS in the formulation of 20vPnC. For the seven new pneumococcal polysaccharide (b) (4) the 20vPnC DP, Pfizer completed sterility and bacterial endotoxin method validations; in addition, the (b) (4) is tested for (b) (4).

This review covers the analytical testing methods and their validation for testing DS MBC serotypes, in process cross reaction material protein CRM<sub>197</sub> and the 20vPnC DP.

## Review Narrative

### 1. (b) (4)

(Kouassi Ayikoe)


(b) (4)

## Method

(b) (4)



(b) (4)




#### **4. Aluminum Concentration in 20vPnC DP (Kouassi Ayikoe)**

Aluminum content in 20-valent pneumococcal conjugate drug product is quantitated by (b) (4)

The drug product release specification is (b) (4)


##### Method

(b) (4)



##### Method Validation

The following characteristics were validated for the method: Specificity, Accuracy, Linearity, Range, Repeatability, Intermediate Precision and Robustness. (b) (4)



Specificity was demonstrated with (b) (4)

which met the acceptance criteria.

Accuracy was determined by (b) (4)

. All

results met the acceptance criteria.

Repeatability was demonstrated by (b) (4)

The acceptance criterion (RSD (b) (4)

Intermediate precision was determined by (b) (4)

All results passed the acceptance criterion of (b) (4)

Linearity for standards was demonstrated by (b) (4)

and met the acceptance criterion of (b) (4) .

Range of the assay method was established to be (b) (4)

. The range of the assay encompasses the DP specification for AI and is therefore suitable.

Robustness of the method was demonstrated during assay development with (b) (4)

which met the acceptance criterion of (b) (4)

Conclusion:

The (b) (4) assay for the quantitative determination of Aluminum adjuvant in 20-valent pneumococcal conjugate drug product and its validation is acceptable and is therefore suitable for its intended purposes.

**5. Total Protein (b) (4) in 20vPnC DP (Kouassi Ayikoe)**

The total protein concentration (b) (4) of 20vPnC DP are determined using a (b) (4) method. The release specifications for total protein concentration is (b) (4). For the system suitability, the Coefficient of determination (b) (4)

Method

(b) (4)



Method Validation

The total protein concentration (b) (4) method for 20vPnC DP has been validated for precision (repeatability, intermediate precision, reproducibility), accuracy, specificity, linearity, range, and robustness.

Specificity of the method was demonstrated by (b) (4)

[REDACTED]

All the acceptance criteria for specificity were met.

For repeatability, the acceptance criteria for total (b) (4)

[REDACTED]

The result met the acceptance criteria of (b) (4)

Intermediate Precision was demonstrated using (b) (4)

[REDACTED]

The result met the acceptance criteria of (b) (4)

Reproducibility was demonstrated by (b) (4)

[REDACTED]

(b) (4) and met the acceptance criteria of (b) (4)

Accuracy of the method was demonstrated (b) (4)

are sufficient to demonstrate the method accuracy.

Linearity of the method was demonstrated for (b) (4)

linearity have been demonstrated.

(b) (4)

as the range for the method is appropriate.

Robustness was part of the method development. (b) (4)

The robustness was demonstrated.

Conclusion:

The validation acceptance criteria, as stated in the protocol, were met, and the analytical procedure was performed in accordance with the requirements of the test method. The total protein concentration (b) (4) analytical procedure is validated and suitable for its intended uses.

**6. Appearance for (b) (4) 20vPnC drug product** (Ritu Agarwal)

(b) (4)

[Redacted text block]

[Redacted text block]

[Redacted text block]

[Redacted text block]

[Redacted text block]

[Redacted text block]

[Redacted text block]

[Redacted text block]

Method for color and opalescence of Drug product (DP)

20vPnC DP test samples (TS) (typically (b) (4))

[REDACTED]

Method verification

(b) (4)

[REDACTED]

The release results of 20vPnC drug product batches used in non-clinical studies, clinical and process validation were acceptable. Visual inspection is appropriate to verify the appearance, color and turbidity; and validation of these methods is not necessary.

Conclusion: The assay is approvable as a release test for (b) (4)  
[REDACTED] 20vPnC drug product.

**7. Determination of (b) (4) 20vPnC drug product** (Ritu Agarwal)

(b) (4)

[REDACTED]



Method

(b) (4)

[REDACTED]

**8. Assay for (b) (4)** (Ritu Agarwal)

(b) (4)





**9. Quantitation of Polysorbate 80 by (b) (4) for 20vPnC drug product (Ritu Agarwal)**

Polysorbate 80 is an excipient in 20-valent Pneumococcal Conjugate (20vPnC) drug product, and its specification is set at (b) (4)

Method

The concentration of polysorbate 80 in drug product is determined by (b) (4)



Method Validation

The characteristics that were evaluated during method validation were: specificity, linearity, accuracy, repeatability, intermediate precision and robustness.

Specificity was established by (b) (4)



(b) (4)

The assessment of linearity of standard was performed by (b) (4)

The accuracy was assessed by (b) (4)

The assessment of method precision was performed by (b) (4)

Based on the evaluation of accuracy, linearity, and precision, range of the method was defined as (b) (4)

The Robustness was evaluated for (b) (4)

Conclusion: The method is clearly described and validated, and is acceptable as a quality control test for the quantitation of polysorbate 80.

#### **10. Volume of injection for 20vPnC Drug product (Ritu Agarwal)**

##### Method

The volume of injection analytical procedure is equivalent to, and complies with, the current (b) (4)

The batch analysis data for 0.5 mL single-dose prefilled syringes, for process validation, engineering, pre-clinical and clinical (phase 3) lots, were as specified. Thus, the method is suitable for testing of 20vPnC drug product.

Information request: The following IR was submitted to the sponsor on 16 February 2021. The response by the sponsor, received on 25 February 2021, is discussed below.

A. Please submit a brief description of the method 'Volume of injection' (section 3.2.P.5.2), which is used as a lot release test for 20vPnC drug product.

Review of response: As requested by CBER, the sponsor provided the description of the method used for measuring 'Volume of injection'. The details are incorporated in the review section above.

Conclusion: This is a well-established method. The assay is approvable as a release test for 20vPnC drug product.

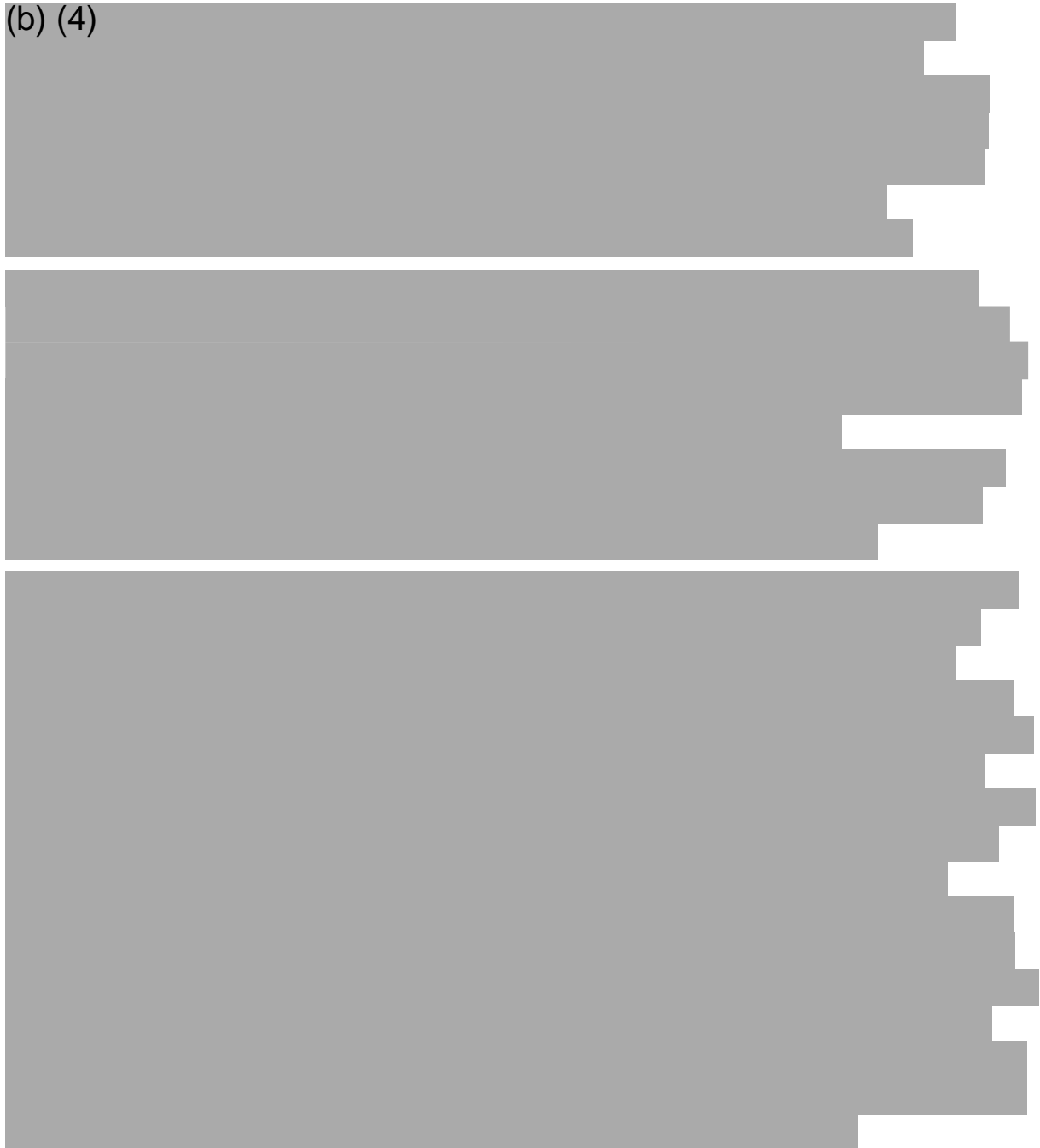
#### **11. Sterility Test Qualification for 20vPnC (b) (4)**

(Hyesuk Kong)

DP

(b) (4)

(b) (4)



#### Sterility Test Qualification for 20vPnC DP

The 20vPnC DP was qualified using the (b) (4) method by performing (b) (4) studies at Pfizer facility in (b) (4) to demonstrate the 20vPnC DP (b) (4).

The (b) (4) sterility test is performed for (b) (4) products in accordance with (b) (4) and provides assurance of sterility. (b) (4)



product sample is added (b) (4)

The method is described below, as the method qualification demonstrates suitability of the test method according to its indicated use.

Pfizer performed the sterility test method qualification using (b) (4)

(b) (4)

#### Information Request for Sterility and Review

The following IR was submitted to the Sponsor on 11 January 2021 and the response was received on 25 January 2021 in amendment 125731/0/10.

1. For sterility method validation by (b) (4) (section 3.2.S.4.3) for (b) (4) (i.e., (b) (4)) please provide the volume of product sample tested in each medium.

2. Sterility method validation by (b) (4) (section 3.2.P.5.3) was performed by (b) (4) the indicator test microorganism specified in (b) (4). The following information is requested to complete its review:
- Since 1 mL syringe contains a 0.5 mL dose of 20vPnC DP and the validation was performed using (b) (4) the amount of product, CBER finds it incomplete and recommends the validation to be repeated using total of 20 syringes per media (i.e., total of 40 syringes) to ensure product does not have any (b) (4) properties and will not inhibit the growth of microorganisms;
  - Please explain/clarify why (b) (4) was used and why (b) (4) was not used for the (b) (4) suitability study
  - Please provide the complete sterility qualification report for DP to include type of media, conformance lot numbers, incubation conditions and duration to show suitability of sterility assay for the intended purpose

#### Review of the Response

Pfizer provided the sample size used in the sterility test and clarified the (b) (4) strain was used as their positive control in the method. In addition, Pfizer provided a detailed report that included the information requested above. This reviewer determined they had all the information required to complete the review of this sterility method and their responses were found acceptable.

#### Conclusion: (b) (4)

The (b) (4) sterility test is performed in accordance with (b) (4) and provides assurance of sterility of the Drug Product. The qualification studies demonstrate this method is suitable for its intended purpose.

(b) (4)

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

(b) (4)

**13. Bacterial Endotoxin Test Method for (b) (4)** DP  
(Hyesuk Kong)

Pfizer qualified both the (b) (4) [REDACTED] for testing the (b) (4) [REDACTED]. CBER asked Pfizer to specify one method; and Pfizer stated their primary method for endotoxin determination for (b) (4) [REDACTED].

(b) (4) is the (b) (4) method and the (b) (4) method will be their alternate method (IR response – Amendment 125731/0/10).

(b) (4) Bacterial Endotoxin Test (b) (4) Method

(b) (4) is performed to quantitate bacterial endotoxins by (b) (4)

[REDACTED]

(b) (4)

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]



(b) (4)

(b) (4)

(b) (4) Qualification for DP

Pfizer qualified their (b) (4) method for the 20vPnC DP at their Pfizer facility in (b) (4), to demonstrate their method is suitable under the actual conditions of use in accordance with (b) (4)

The MVD for the DP was calculated to be (b) (4)

(b) (4)

. This reviewer finds the selected testing acceptable.

Pfizer submitted bacterial endotoxin concentration results (b) (4) for several DP lots and all were found to be within their proposed release specification of not

more than (b) (4). CBER finds their proposed (b) (4) release specification of (b) (4) acceptable.

#### Information Request for Bacterial Endotoxin Test Method and Review

The following IR was submitted to the Sponsor on 11 January 2021 and the response was received on 25 January 2021 in amendment 125731/0/10.

5. In summary data of the test for interfering factor results for (b) (4) (sample types: (b) (4)) presented in Table 3.2.S.4.3-3 thru Table 3.2.S.4.3-5, all endotoxin concentration lot results were below (b) (4). The following information is requested to complete its review:

- Please provide the calculation and figures used to determine the endotoxin concentration result below (b) (4)
- Please provide test results as EU/mL including the converted to EU/μg as the specification is (b) (4); and
- Please show in detail how maximum valid dilution was calculated for (b) (4)

6. In your test results of interfering factors for 20vPnC DP (section 3.2.P.5.3), all tested dilutions (b) (4) showed acceptable inhibition or enhancement. Please provide your final selected testing dilution for your routine release testing.

#### Review of the Response

Pfizer clarified the endotoxin concentration calculations and updated MVD calculation in the response. The response was found acceptable.

Based on the data reviewed, the (b) (4) the 20vPnC DP did not exhibit any inhibitory or enhancement factors that would adversely impact the (b) (4) test. After review of (b) (4) -BET method qualification results and details provided in the IR responses, this reviewer concludes these test methods were qualified and performed in accordance with (b) (4)

#### Conclusion

After a thorough review of the information submitted this reviewer finds Pfizer's endotoxin test method qualifications were performed in accordance with (b) (4) and determined the results provide evidence of method suitability under the actual conditions of use. Therefore, this reviewer finds the (b) (4) BET



method to test all (b) (4) for testing the (b) (4), DP (b) (4) acceptable.

#### **14. Review of Validation of Identity (ID) Assay for (b) (4) DP (Anil Choudhary)** Historical Perspective

Pfizer's Prevnar13 DP was licensed in 2010 (STN 125324). Prevnar 13 contains serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F conjugated to CRM<sub>197</sub> (Cross-Reactive Material, nontoxic carrier protein of Diphtheria toxin (DT)). DBSQC reviewed the Identity assays (STM-I-1006, 13V-GMT-0006 and STM-00004126) and validation of the Identity test method for 13 Pneumococcal polysaccharides serotypes and CRM<sub>197</sub> of Prevnar 13. The Identity (ID) test method was found suitable for intended purpose of detection of the 13 serotypes of Pneumococcal polysaccharides and carrier protein. In the current submission, sponsor has added seven (7) more Pneumococcal polysaccharides serotypes- 8, 10A, 11A, 12F, 15B, 22F, and 33F (called c7V) to a total of twenty (20) serotypes in the final product Prevnar 20 conjugated to CRM<sub>197</sub> protein.

#### Method Overview

The sponsor provided three SOPs in response to DBSQC IR (dated January 21, 2021). The SOPs- #13496, #LAB-4663 (previously known as 13V-GMT-0006) and #32763 (previously known as STM-I-1006) describe the method for identification of Pneumococcal polysaccharides and carrier protein (Diphtheria CRM<sub>197</sub> protein) by (b) (4). All the three procedures are basically similar, except that different (b) (4)/DP are tested using their respective antibodies. Below are the different scopes of the three SOPs;

SOP #13496 (used at (b) (4) facility of Pfizer at (b) (4) and SOP# LAB-4663 are used for the following different products: (b) (4)


Drug

Products that are multivalent (13V, c7V, 20V, 13V Comparator) vaccine formulations, and in-process or filled syringes/vials.

SOP#32763 applies to testing of (b) (4)

Briefly, antiserum specific to each Pneumococcal polysaccharide and the carrier protein are used to determine the presence or absence of serospecific polysaccharides and carrier proteins in the test sample. As per the scope of the test method, appropriate controls are included to test the specificity of each test run. This method is an (b) (4)

(b) (4)



Review of Validation of Identity (ID) Assay for (b) (4) DP

The validation of the Identity test methods was performed in conformance with ICH Q2(R1) guidelines, for establishing the “Identity” of the Pneumococcal polysaccharide serogroups and carrier protein (Diphtheria CRM<sub>197</sub> protein). The ICH guidelines require the assessment of the specificity parameter only for validation of a method used for ID of (b) (4) / product.

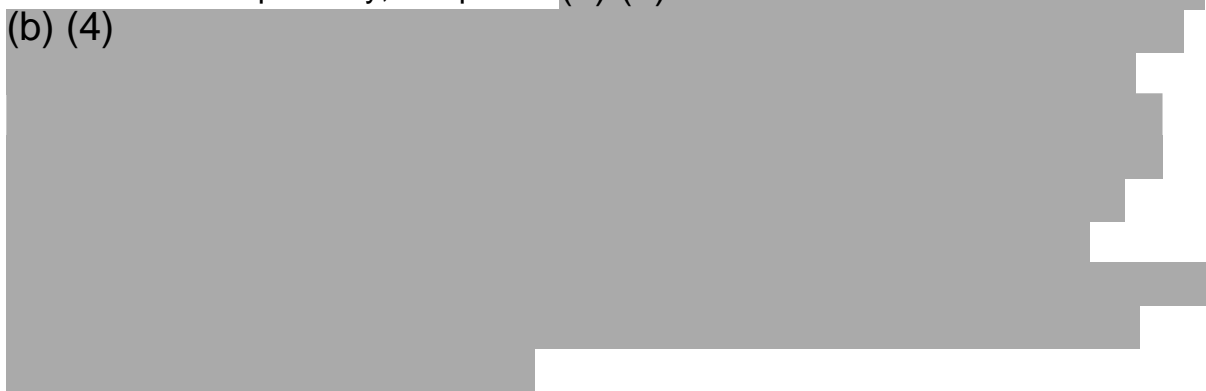
Sponsor has provided summary documents in support of ID validation for (b) (4) under section 3.2.S.4.3. Under section 3.2.P.5.3, summary of ID validation has been provided for 20-vPnC DP for specificity along with the robustness (b) (4)

To assess ID, following type specific antibodies for component antigen were used;

- Monoclonal anti-serotype 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, and 33F polysaccharide.
- (b) (4) anti-Diphtheria CRM<sub>197</sub> protein.

To demonstrate specificity, samples of (b) (4)

(b) (4)





The results of all robustness assays met the predetermined acceptance criteria.

### Conclusion

The sponsor provided the summary documents for validation of identity assay for (b) (4) DP to demonstrate the evaluation of specificity of the method and robustness for (b) (4). As per ICH Q2(R1) guidelines, the specificity parameter is the only parameter needed to be assessed to demonstrate the validation of an identity test method. The results in the validation report summaries demonstrate that the specificity parameter evaluated for (b) (4) DP met the predefined specification. The results from robustness evaluation was performed by demonstrating (b) (4) for testing DP and met the specifications. The assays provided by the sponsor for demonstrating the Identity of the Pneumococcal polysaccharides and carrier protein CRM<sub>197</sub> were validated appropriately. The test methods are suitable for the intended purpose of testing the identity of Pneumococcal polysaccharides in (b) (4) 7-valent, 13-valent and 20-valent DP.