



**MEMORANDUM**

**Date:** February 12, 2010

**To:** STN 125324/0

**From:** Rajesh K. Gupta, Ph.D., HFM-407  
Deputy Director, Division of Product Quality (DPQ) and  
Lab Chief, Product Quality Laboratories

**Through:** William McCormick, Ph.D., HFM-407  
Director, Division of Product Quality (DPQ)

**Subject:** STN 125324: — Pneumococcal Saccharide Conjugated Vaccine Adsorbed,  
13-valent, Prevnar 13, Review of Drug Substance and Drug Product  
Analytical Procedures

**CC:** Julienne Vaillancourt  
Michael Smith, Ph.D.  
Milan Blake, Ph.D.  
Willie Vann, Ph.D.  
John Cipollo, Ph.D.  
William McCormick, Ph.D., HFM-407

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Review of the analytical procedures and the associated validation protocols and reports was performed by the staff of Division of Product Quality (Reviewers from DPQ: Rajesh K. Gupta, Alfred Del-Grosso, James Kenney, Manju Joshi, Muhammad Shahabuddin, Karen Campbell, Hsiaoling Wang, Nora Etz, Joe Progar, and Brandon Duong). Specifications for methods used to release Drug Substance and Drug Product were also reviewed.

**SUBMISSIONS REVIEWED**

STN 125324/0.3, Sections 3.2.S.4 (for serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F), and 3.2.P.5  
STN 125324/0.15 (amendment received June 11, 2009)  
STN 125324/0.22 (amendment received July 23, 2009)  
STN 125324/0.31 (amendment received September 21, 2009)  
STN 125324/0.37 (amendment received October 8, 2009)  
STN 125324/0.40 (amendment received October 12, 2009)  
STN 125324/0.44 (amendment received October 16, 2009)

STN 125324/0.47 (amendment received October 28, 2009)  
STN 125324/0.48 (amendment received November 5, 2009)  
STN 125324/0.51 (amendment received November 20, 2009)  
STN 125324/0.54 (amendment received November 24, 2009)  
STN 125324/0.55 (amendment received November 24, 2009) – Information on the number of lots to be submitted after licensure, Not cited in this review memo  
STN 125324/0.61 (amendment received December 8, 2009)  
STN 125324/0.64 (amendment received on December 11, 2009)  
STN 125324/0.71 (amendment received on January 11, 2010)  
STN 125324/0.79 (amendment received on February 3, 2010) – Copies on Materials presented by Wyeth in meetings on October 5, 2009 and November 5, 2009 with regard to in-support testing for -----b(4)----, Not cited in this review.  
STN 125324/0.83 (amendment received on February 10, 2010)

**METHODS REVIEWED**

Drug Substance

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- Sterility Test by ---b(4)-----

Drug Product

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- Aluminum --b(4)-----  
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- Endotoxin --b(4)-----

- Identity, Polysaccharide and CRM<sub>197</sub> by -b(4)----
- Polysorbate 80
- Protein – --b(4)----- by modified --b(4)-----
- General Safety
- Sterility Test by --b(4)-----

**RECOMMENDED ACTION**

The data submitted to support the analytical methods used for testing of Drug Substance and Drug Product of Pneumococcal Saccharide Conjugated Vaccine Adsorbed, 13-valent, Prevnar 13, were reviewed and a number of issues with regard to adequacy of method validations and the lack of a test for the active ingredient (conjugate) in the DP were found. Specifications for --b(4)----- tests performed on the Drug Substance (DS) were wider than the data from lots used in the clinical trials. A number of post-marketing commitments (listed below) were generated to ensure consistency in manufacture of the product and consistent performance of analytical methods. With these post-marketing commitments, I recommend approval of this application.

**REVIEW SUMMARY AND POST MARKETING COMMITMENTS**

Analytical procedures and the associated validation protocols and reports for the Drug Substance and Drug Product were reviewed. A number of concerns and questions about the methods in the original submission were communicated to the sponsor. During the BLA review process, the sponsor submitted amendments providing clarifications and additional documentation. There still remain a number of outstanding issues, which CBER requests be addressed according to timelines agreed upon by the sponsor. In an amendment 0.83 received on February 10, 2009, sponsor has agreed to address issues related to methods validation. These post-marketing commitments related to analytical methods are listed below.

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**DETAILED REVIEW AND COMMENTS**

Wyeth provided summaries of methods and method validations in the biologic license application. In order for CBER to perform a complete review and to perform testing in support of the BLA, it was communicated to Wyeth on April 30, 2009 to provide details of methods, copies of validation protocols, and validation reports for all methods used for release testing of Drug Substance and Drug Product. After a tele-conference with Wyeth on May 5, 2009, Wyeth provided all the methods by Email on May 7, 2009, and provided copies of Global Test Methods, validation protocols and reports on 2 CDs on May 21, 2009. Global test methods, validation protocols and validation reports were also submitted in amendment 0.15 on June 11, 2009.

**A. General Comments on Methods, Method Validations and Specifications**

i. Use of a Standard Curve with --b(4)-----points in Quantitative Assays

A standard curve with --b(4)----- points generated by linear regression has been used for the following methods.

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Standard curves for calculation of results of unknown samples in quantitative analytical methods usually contain b(4) or more points, except certain immunochemical methods, where the response is not linear. This is a basic concept in standardization and method development. The sponsor has demonstrated linearity of standard curves using b(4) points in validation studies. Validation using a b(4) point standard curve is not an acceptable alternative or justification for using other than a scientifically sound standard curve with enough points in every assay. Further, demonstration of linearity of a standard curve during method validation is not acceptable as a proof of linearity for the method. We ask Wyeth to develop all quantitative methods to use a b(4)-point standard curve.

**Wyeth’s Response (Amendment 0.47)**

Wyeth believes that the use of b(4) point curves for routine analytical analyses is appropriate and in common use in the pharmaceutical industry. Wyeth follows -b(4)--- guidelines for analytical validation. The supporting data and explanation for Wyeth’s position is described in detail in the document titled “-----b(4)-----



## DPQ's Response

We have reviewed sponsor's response, and supporting data and explanation submitted in section 1.11.1 of amendment 0.47. We do not agree with the concept of b(4) point standard curve for all analytical methods for the following reasons.

- a. The principles of the AOAC International are applicable to the AOAC methods. None of the methods described here are described by AOAC.
- b. -b(4)- point standard curves should not be used for critical methods for a product, in this case, the --b(4)----- assays, results from which are used to formulate vaccine, and --b(4)----- which evaluate critical parameters of the product to ensure the quality of the product and consistency in manufacture. This is particularly important for this product, where no direct method is available to measure the active ingredients (conjugate) in the final formulated and final fill products. Having a scientifically sound standard curve for these methods, -b(4)---assay, --b(4)----- assay, -----b(4)----- method, is important to ensure acceptable accuracy and precision of results generated.

The approach of using --b(4)-----point standard curve may be acceptable for certain methods used to verify amount of excipients and to measure residuals from the process, where specifications are wider than actual results. Based on this, we accept b(4)-point standard curves for the --b(4)----- method and --b(4)----- method, but a b(4) point standard curve should be included for the ---b(4)----- method.

## Conclusion

Wyeth commits to modify the following methods to incorporate the use of a b(4) point standard curve with each test.

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- ii. Lack of a test for active ingredient (serotype specific polysaccharide-protein conjugate) and lack of an assay that is appropriately stability indicating on the Drug Product

There is no test for detection of polysaccharide-protein conjugate for any of the 13 serotypes on the Drug Product.

In a tele-conference on June 19, 2009 and subsequent face-to-face meeting between CBER and Wyeth on September 1, 2009, CBER requested that Wyeth develop a test for conjugate at the Drug Product stage that can be used as a stability indicating assay for the individual conjugates. CBER and Wyeth agreed that the scientific principle should be demonstrated on two pneumococcal conjugates, one being representative of the -b(4)----- and the other being representative of the -b(4)--- conjugation process (amendment 0.48 received on November 5, 2009).

In the amendment 0.37 received on October 8, 2009, Wyeth submitted studies in support of the --b(4)----- assay as performed by a -b(4)----- method to test for presence of conjugate in the Drug Product and also as a stability indicating method for 11 of the 13 serotypes based on the data from two representative pneumococcal conjugates. The -b(4)----- assay “Quantitation of ---b(4)----- in 13vPnC Pneumococcal Vaccine in Polysorbate 80 Formulation by -b(4)-----” is used to quantify, in a serotype-specific manner, --b(4)----- of each of the polysaccharides in the 13vPnC. The --b(4)-----  
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**DPQ’s Comments**

Based on the information contained in RPT-69871, and amendments 0.22 and 0.37, there is not enough evidence that the --b(4)----- method, as presented, measures only the conjugate in a consistent manner and that this assay meets criteria considered appropriate as a stability indicating method. Scientific principle or proof of concept that this method is a stability indicating has not been demonstrated.

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**Conclusion**

Based on the information contained in RPT-69871, and amendments 0.22, 0.37, 0.61, and 0.64 and discussions during December 3, 2009 tele-conference, Wyeth has not provided enough evidence that the -b(4)-----  
-----method, as presented, measures only the conjugate in a consistent manner and meets criteria considered appropriate as a stability indicating method. Scientific principle or proof of concept has not been presented to show that this method is stability indicating.

Wyeth agrees to develop and validate a scientifically sound stability indicating assay, which may not be limited to the --b(4)-----  
-----method, for polysaccharide protein conjugate content for each of 13 serotypes in the Drug Product. This assay will be used for release and stability testing of the Drug Product. Wyeth will work closely with CBER in developing this method and provide quarterly reports on the progress.

iii. Specifications at Drug Substance

Wyeth’s specifications for --b(4)-----  
----- for DS were wider than the results for these parameters from the lots used in clinical trials, especially for 6 additional serotypes added to Prevnar (1, 3, 5, 6A, 7F, and 19A) and 19F. On October 7, 2009, CBER requested updated license application documents for the updated specifications for monovalent bulk conjugate (MBC) for serotypes 1, 3, 5, 6A, 7F, 19A and 19F.

**Wyeth’s Response (amendment 0.44 received on October 16, 2009)**

This amendment provides the specification and justification of specification documents for MBC for serotypes 1, 3, 5, 6A, 7F, 19A and 19F and the Drug Product. Wyeth has reviewed additional recent production data, the stability profiles of clinical batches, and the attribute data from conjugates used in related clinical programs. The conjugate attributes specifically evaluated are:

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----- As a result of these reviews, the additional analyses of data, and particularly in consideration of the available clinical experience, we are now proposing more stringent specifications for most of these attributes. Additionally, Wyeth added general safety test to the DP. These changes have been incorporated into the appropriate dossier documents.

### **DPQ's Response**

We agree with the updated specifications for --b(4)----- for types 1, 6A and 7F. Further evaluation is required for --b(4)----- specifications for type 3 (stability), type 5 (both release and stability), type 19A (stability) and type 19 F (stability, widened from --b(4)-----

We agree with --b(4)----- specifications for types 3, 5, 6A, 7F and 19A. Further evaluation is required for --b(4)----- specifications for type 1 (stability), and type 19F (stability, widened from --b(4)-----

### **Conclusion**

Wyeth commits to re-evaluate the --b(4)----- specifications after accruing values from -b(4)- lots of Drug Substance or through the end of June 2012, whichever is sooner.

#### iv. General Comments on Methods Validations

In all method validation documents, Wyeth has referred to ICH guidelines Q2(R1) for evaluation of various validation parameters for methods used in testing of DS and DP. ICH, the Q2(R1) Guideline states that the specified range of an assay "...is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of analyte within or at the extremes of the specified range of the analytical procedure." Wyeth has not evaluated linearity, accuracy and precision for all quantitative methods across the range of the method. This was communicated to the sponsor in a tele-conference on June 19, 2009 as Question 3 given below.

Question 3. The validations should be performed according to ICH guidelines. For example linearity, accuracy and precision of the methods for most validations reviewed thus far do not contain data on entire range of method, accuracy and precision are not evaluated on reportable results, and linearity is not evaluated with samples.

Subsequent to the tele-conference on June 19, 2009, Wyeth submitted amendments 0.22 and 0.31 explaining the evaluation of various validation parameters for all quantitative methods used in testing of DS and DP. These amendments re-evaluated data for intermediate precision to include only reportable results, but did not address the following issues.

- a. Demonstration of an acceptable level of linearity, accuracy and precision over the specified range of the method using DS and DP samples.
- b. Demonstration of linearity, and accuracy for reportable results, as defined in applicable Test Method documentation.
- c. Evaluation of accuracy by spiking DS and DP with appropriate test materials, not heterologous standards. In performing spiking studies, accuracy should be evaluated based upon recovery of quantity spiked into appropriate DS and DP samples and not from total amount of analyte determined in sample (sample content plus spike) and reportable results should be presented (as defined in Test Method documentation) or weight-per-volume measure in addition to % spike recovery.
- d. Evaluation of linearity from final results (not raw data) using samples, not from data derived by testing of standards.
- e. For intermediate precision, data should be presented at individual levels, not normalized by multiplying with the dilution factor.

These issues were further discussed with Wyeth in a tele-conference on December 1, 2009 and Wyeth agreed to evaluate various validation parameters, as discussed above. Method specific post marketing commitments (PMC) have been generated to address these issues.

- v. Discrepancy in Document IDs for Methods Validated versus Methods Used in Testing

Test methods submitted for the following assays have document IDs different from the document IDs cross-referenced in the corresponding validation documents. We ask Wyeth to confirm that the method you validated is the one currently used in testing of DS and DP. We ask Wyeth to provide a complete list of validation documents, a list of test methods referred in these documents and a list of test methods currently used (provided in the submission) highlighting and explaining any differences

or changes in these methods as referred in the corresponding validation documents and those currently used. Please see examples below.

--b(4)-----, Test method referred to in validation documents STM-C-7138 Rev 4.0

Test method submitted in BLA STM-C-1101

**Free Saccharide**, Test method referred to in validation documents STM-C-7026 Rev 5.0/6.0

Test method submitted in BLA STM-C-1075

**Saccharide -----b(4)-----**, Test method referred to in Validation Documents STM-C-1062

Test Method submitted PRCS-0035.

**Total Protein (--b(4)-----) Procedure and --b(4)----- Protein Determination**, Test Methods referred to in validation documents STM-C-7058 and STM-C-7075

Test Method submitted in BLA STM-C-1016 and STM-C-1073 and 13V-GTM-0013 (--b(4)---- proteins)

--b(4)----- **by Modified --b(4)-----**, Test Methods referred to in validation documents STM-C-7169

Test Method submitted in BLA STM-00004124 and 13V-GTM-0044

**Polysorbate 80 by --b(4)-----**, Test Methods referred to in validation documents STM-C-7159

Test Method submitted in BLA STM-00004127.

This was communicated to Wyeth in a tele-conference on December 1, 2009 and by an Email as Question 1 on December 16, 2009. Wyeth agreed during the tele-conference on December 1 to submit a genealogy of the documents.

### **Wyeth's Response (amendment 0.71)**

The Analytical Development and QC laboratories are within different organizations (Development vs. Pfizer Global Manufacturing); the SOPs are named and numbered differently between the groups. The Analytical Development SOPs can be distinguished from the site based QC laboratory methods based on the SOP number format. All of the Analytical Development SOPs are numbered following the pattern of



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ii. Sterility Test by --b(4)-----

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Documents reviewed, STM-M-1003, 13V-GTM-0051, 13V-GTM-0051-AVS, GCV-P-1837-01, Q-M-1003-06-Pneumo 3 & 19A, GCV-P-1846-01, GCV-P-2230-00, GCV-P-2287-00, GCV-P-1915-02, Q-M-1003-06-Pneumo 4, 18C, 9V, 14, 23F & 6B, GCV-R-2421, Q-M-1003-06-Pneumo 3& 19A Revision Level A, GCV-R-2731, GCV-R-3081, GCV-R-3039, GCV-R-2674, Q-M-1003-06-Pneumo 14, Q-M-1003-06-Pneumo 18C, Q-M-1003-06-Pneumo 23F, Q-M-1003-06-Pneumo 4 & 9V and Q-M-1003-06-Pneumo 6B

**DPQ's Comments**

The method is adequate for intended purposes.

**C. Specific Comments on Methods for Drug Product**

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Documents reviewed, STM-00004125, 13V-GTM-0046, 13V-GTM-0046-AVS, RPT-68036 and RPT-69871.

**DPQ's Comments**

- a. Total -----b(4)-----measures total saccharide in Drug Product. This method can not distinguish between -----  
--(b)(4)-----This was communicated to Wyeth during a tele-conference on June 19, 2009 as a following question.

Which assay does the firm consider as proof of conjugation or quantitation of the amount of conjugate?

**Wyeth's Response (Amendment 0.22)**

Proof of conjugations is not determined by a single assay, but instead a series of assays, which demonstrate maintenance of the large molecular size of the conjugated saccharide, limited amounts of free (unconjugated) protein and defined amounts of free (unconjugated) saccharide after the conjugation reaction. The -b(4) main assays are the --b(4)-----  
-----assay.

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- b. We have concerns regarding the use of --b(4)-----  
----- method.  
-----b(4)----- used for the --b(4)----- method should be raised  
against a -----b(4)-----  
-----b(4)--, preferably using a different chemistry, if possible.

This was discussed with Wyeth in a tele-conference on June 19, 2009. Wyeth submitted a detailed response with data in amendment 0.22 --b(4)-- -----  
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----- test under the conditions of the method. Since total --b(4)----- is not a suitable method to quantitate active ingredient, serotype specific polysaccharide-protein conjugate, in Drug Product, this issue was not pursued further.

- c. Intermediate Precision: we ask Wyeth to provide intermediate precision data separately for each day and each analyst as reportable results. Replicate data obtained from the same analyst on the same day should not be included in the evaluation of intermediate precision.

**Wyeth's Response (amendment 0.22)**

Wyeth re-analyzed the intermediate precision data removing the nested repeatability, which has been presented in Tables 3-59 through 3 -61 in Module 1 submitted in amendment 0.22.

**DPQ's Review of Wyeth's Response**

Wyeth's response is adequate with regard to data analysis for intermediate precision.

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**DPQ’s Review of Wyeth’s Response**

The response is adequate.

- c. Intermediate Precision: we ask Wyeth to provide intermediate precision data separately for each day and each analyst as reportable results. Replicate data obtained from the same analyst on the same day should not be included in the evaluation of intermediate precision.

**Wyeth’s Response (amendment 0.31)**

Wyeth re-analyzed the intermediate precision data removing the nested repeatability, which has been presented in Table 1-6 in Module 1.11.1 submitted in amendment 0.31.

**DPQ’s Review of Wyeth’s Response**

Wyeth’s response is adequate with regard to data analysis for intermediate precision.

- iii. Endotoxin by --b(4)--- -----

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Documents reviewed, STM-00004127, 13V-GTM-0045, 13V-GTM-0045-AVS, RPT-67842 and RPT- 69364

### DPQ's Comments

- a. We ask Wyeth to specify or evaluate the procedural range for this method based on acceptable precision, accuracy and linearity of the method.

Wyeth commits to define the range of the Polysorbate 80 assay from --b(4)----- Wyeth has already provided data on precision, accuracy and linearity to support the working range for the method. Wyeth commits to re-present data to support the defined range.

- b. As presented in STM-00004127- version 2.0 (Section VI. 4 b) and 13V-GTM-0045 (Section 9.0 – 5), the control is tested in --b(4)-----, whereas samples are tested --b(4)-----

Wyeth commits to determine the control and test sample measurements in the same manner and this shall be in --b(4)-----  
Wyeth commits to revise the procedure to include ---b(4)-----  
Measurement of the control sample

- c. Assay system suitability criteria are described in 13V-GTM-0045 (Section 13.0 – 2.0) and STM-00004127- version 2.0 (Section IX. D. 2 – 4) as %RSD of the system suitability for standard, consistency of --b(4)----- of additional system suitability --b(4)----- and slope, intercept and coefficient of determination of the standard curve.

Wyeth commits to revise the method to include --b(4)----- and a measure of --b(4)----- such as -b(4)----- factor or --b(4)----- as part of the system suitability. In this regard, Wyeth may consult --b(4)----- General Chapter --b(4)----- which describes measures for --b(4)----- performance for system suitability.

- d. Intermediate Precision: we ask Wyeth to provide intermediate precision data separately for each day and each analyst as reportable results. Replicate data obtained from the same analyst





Documents reviewed, STM-00004054, 812-08-050-P.0, 07-01-065-4.0, 07-01-078-1.0, 07-02-001-20.0, 812-08-050-R.0, 07-02-F002-20.0 and 07-02-F029-3.0

**DPQ's Comments**

Wyeth provided both options of --b(4)-----  
for the sterility test of Drug Product. We ask Wyeth to specify one method that has been qualified for bacteriostasis and fungistasis with the product matrix. This was communicated to Wyeth during tele-conference on December 1, 2009.

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**Conclusions**

Wyeth's response is adequate. --b(4)--- -----  
----- . The method is suitable for intended purposes.

x. Test for Succinate

The Prevnar 13 formulation has 5 mM succinate buffer. The effect of succinate on the adsorption and physico-chemical characteristics of aluminum phosphate adjuvant is not described in the application. Succinate content of the Drug Product was evaluated for the clinical trial lots, but this test was discontinued for the commercial product. Since succinate is not a common excipient used in the formulation of vaccines and may have an effect on adsorption of conjugates to the aluminum phosphate adjuvant, quantitation of succinate at Drug Product is required.