# DBSQC/OCBQ ANALYTICAL METHOD REVIEW MEMO

**To:** The file: STN 125742/0

**From:**

| Reviewer | Date finalized | Stamp | Supervisor | Stamp |
|---|---|---|---|---|
| Emnet Yitbarek (Lead reviewer) | 8/20/2021 | | Kori Francis | |
| Hsiaoling Wang | 8/19/2021 | | Tao Pan | |
| Esmeralda Alvarado-Facundo | 8/20/2021 | | Muhammad Shahabuddin | |
| Anil Choudhary | 8/20/2021 | | | |
| Karla Garcia | 8/20/2021 | | James Kenney | |
| | | | Simleen Kaur | |

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

Mary A. Malarkey, Director CBER/OCBQ

**Applicant:** BioNTech Manufacturing GmbH

**Subject:** Review of Analytical Methods used for the Lot release of COMIRNATY Drug Substance (DS) and Drug Product (DP)

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**Recommendation:** Approval

**Summary:**
The following analytical methods used for lot release of COMIRNATY and the associated method validations or qualifications, were reviewed:

1. RNA (b) (4) DP by (b) (4) (Emnet Yitbarek)
2. Identification and quantification of lipids in DP by (b) (4) (Emnet Yitbarek)
3. (b) (4) (Hsiaoling Wang)
4. (b) (4) (Hsiaoling Wang)

5. General (b) (4) methods to test (b) (4) DP: Appearance, Particulate Matter, (b) (4) (Hsiaoling Wang)
6. Identity of (b) (4) DP by (b) (4) Assay (Esmeralda Alvarado-Facundo)
7. (b) (4) (Esmeralda Alvarado-Facundo)
8. (b) (4) (Esmeralda Alvarado-Facundo)
9. (b) (4) (Anil Choudhary)
10. (b) (4) (Anil Choudhary)
11. (b) (4) (Anil Choudhary).
12. (b) (4) (Karla Garcia)
13. Endotoxin of (b) (4) DP (Karla Garcia)
14. Sterility of DP (Karla Garcia)

**Conclusion:** The analytical methods and their validations and/or qualifications reviewed for the COMIRNATY drug substance and drug product were found to be adequate for their intended use.

**Documents Reviewed**
Information in sections of the original BLA (STN125742) and IND19736 submissions that describe control of DS and DP (3.2.S.4 and 3.2.P.5, respectively), including descriptions of DS and DP specifications, analytical procedures of DS and DP and validation of these analytical procedures were reviewed. Additional information in amendments specified by each reviewer were also reviewed.

**Background**
On May 6, 2021, Pfizer/BioNTech (sponsor) submitted an original rolling BLA, STN 125742, for COMIRNATY (BNT162/PF-07302048), a prophylactic vaccine for the prevention of COVID-19 which is caused by SARS-CoV-2 virus. COMIRNATY is a white suspension solution composed of mRNA that encodes for the spike protein of the SARS-CoV-2 virus and is encapsulated in a lipid nanoparticle (LNP). The concentrated suspension is stored at -60 °C and is diluted with a sterile 0.9% Sodium Chloride solution, USP, to administer intramuscularly to individuals ≥16 years of age. The proposed dosage is a 30 µg regimen of two 0.3 mL doses given 3 weeks apart.

DBSQC reviews BLAs and their supplements to ensure analytical methods are appropriately described, validated and suitable for the intended purposes. The following facilities perform the methods reviewed:

1. Pfizer Biotherapeutics Pharmaceutical Sciences Analytical Research & Development, Chesterfield, MO (ARD-STL)
2. Pfizer Biotherapeutics Pharmaceutical Sciences Analytical Research & Development, Andover, MA (ARD-AND)
3. Pfizer Global Supply, Kalamazoo, MI (PGS-KZO)
4. Pfizer Global Supply, Andover, MA (PGS-AND)
5. Pfizer Global Supply, Puurs, Belgium (PGS-Puurs)
6. Pfizer Global Supply, Grange Castle, Ireland (PGS-GC)
7. 

The following analytical methods used for DS and DP release were reviewed:

1. **RNA** (b) (4) **DP by** (b) (4)

Method description

(b) (4)

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(b) (4)

Conclusion

The (b) (4) method is adequately validated for the determination of product (b) (4) in COMIRNATY DP (b) (4). The firm's test method includes limited instructions for (b) (4). They have agreed to add the information and submit the updated procedure to their IND.

**2. Identification and quantification of lipids in DP by** (b) (4)

Method description

The identity and quantity of the four lipids in the DP, ALC-0159, cholesterol, DSPC and ALC-0315, are determined by (b) (4). In this method, (b) (4)

The assay system suitability criteria include:

(b) (4)

Assay and DP sample acceptance criteria include:

(b) (4)

Method Validation:

(b) (4)

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(b) (4)

(b) (4)

Conclusion:

The (b) (4) method is adequately described and validated for the identification and quantitation of four lipids (ALC- 0159, cholesterol, DSPC and ALC-0315) in COMIRNATY DP.

(b) (4)

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(b) (4)

**4.** (b) (4) **of DP by** (b) (4)

The DP is a sterile (b) (4) of RNA-containing lipid nanoparticles (LNPs) in phosphate buffer containing sucrose. The specifications of LNP (b) (4) for DP are (b) (4) , respectively. The specification for the (b) (4) .

Method description

(b) (4)

(b) (4)

(b) (4)

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(b) (4)

Conclusion
Based on the information provided in the original BLA and the amendment, the method has been validated for its intended purpose.

**5. General (b) (4) Methods to test (b) (4) DP**

(b) (4) methods used for (b) (4) DP analyses are shown in Table 7 below.

Table 7. (b) (4) methods used to test (b) (4) DP

| (b) (4) **DP** | **Test** | **Specification** | (b) (4) **method** |
|---|---|---|---|
| (b) (4) | | | |
| DP | Appearance: Visual and particles | White to off-white suspension; May contain white to off-white opaque, amorphous particles | (b) (4) |
| DP | (b) (4) | (b) (4) | |
| DP | (b) (4) | | |
| DP | (b) (4) | | |
| DP | Container content | Not less than (b) (4) | |

(b) (4)

These methods do not require validation. Suitability of the methods for this (b) (4) DP was verified as described below.

Review of Appearance Methods and Method Verifications:

The appearance of (b) (4) includes the tests for (b) (4). The appearance tests described in TM100010539 for ARD labs and in LAB-(b) (4) for PGS-AND lab are equivalent.

(b) (4)

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(b) (4)

Review of Container Content and Method Verifications:

The container content is an adaption of the (b) (4) for DP. Analytical procedures in TM100011129 for ARD labs, LAB-(b) (4) for PGS-Puurs and TM9106A for PGS-KZO are equivalent. (b) (4) DP vials are used and (b) (4)

(b) (4)

The container content is calculated to (b) (4) decimal places and every one of the (b) (4) vials should have a volume of no less than (b) (4) to be reported as “No Less Than (b) (4) ”.

(b) (4)

Information Request and Reviews:
The following IR was sent to the firm on August 2, 2021:

a. You calculate volume of each vial based on (b) (4) . Please describe how (b) (4) was determined.
b. In the verification report [(b) (4) ] from PGS-KZO lab, DP container content was determined by measuring the total volume after 1.8 mL of sterile 0.9% sodium chloride solution was added. Please confirm that this method will be used for lot release testing by the PGS-KZO laboratory and that the container volume specification “Not less than (b) (4) ” is the same regardless of test site/method.

Review of the response:
The response was received on August 9, 2021 in amendment 35.

a. The firm stated that the was obtained by
b. The firm confirmed that the container content specification of Not less than is the same regardless of test site/method. Container content for was used as a DP specification under EUA, which was replaced by

vial container content in BLA. PGS-KZO will perform DP container content test as other labs described in analytical procedure TM9106A.

The responses are acceptable. Re-verification from PGS-KZO is not necessary because the analytical steps are equivalent.

Conclusion

Based on the information provided in the original BLA and the amendment, these (b) (4) methods have been verified for their intended purposes.

### 6. Identity of (b) (4) DP by (b) (4) Assay

The (b) (4) assay is a release test to verify the identity of (b) (4) DP by demonstrating the presence of the mRNA target. The test is performed at Pfizer facilities PGS-GC, PGS-AND, ARD and (b) (4). The specification for (b) (4) DP is "Identity confirmed".

Method description

(b) (4)

The assay validity criteria are:

(b) (4)

If the assay fails to meet predefined acceptance criteria, then the assay is invalid and must be repeated. If the DP assay acceptance criteria fail, the sample is re-processed from the beginning (b) (4) .

The sample acceptance criteria are:

(b) (4)

If the assay acceptance criteria and sample acceptance criteria are met, the result is reported as “Identity Confirmed” for the presence of target mRNA or “Identity not confirmed” for the absence of target mRNA.

Method Validation

(b) (4)

(b) (4)

(b) (4)

Conclusion:
The (b) (4) method to confirm the identity of (b) (4) DP mRNA was adequately described and the validation data demonstrate the assay is suitable for its intended use.

(b) (4)

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- (b) (4)
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**10. Total** (b) (4) **RNA by** (b) (4)

The principle of the method to quantify RNA is (b) (4)

Method Description

(b) (4)

(b) (4)

Assay acceptance criteria are as follows:

(b) (4)

Sample acceptance criteria are as follow:

(b) (4)

Reporting of the Results**:** The total RNA content is reported for each test sample in (b) (4) ; the (b) (4) RNA is reported as a whole number. The current specifications for DP are: (b) (4) .

Method Qualification

(b) (4)

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(b) (4)

[redacted]

Overall Conclusion

The RNA (b) (4) method is adequately described and demonstrated to be suitable for measuring total RNA and (b) (4) .

(b) (4)

[redacted]

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### 13. Bacterial Endotoxin Test (BET)

Pfizer qualified the (b) (4) BET method at the ARD-AND and PGS-AND facilities for testing of BNT162b2 mRNA (b) (4). In addition, the (b) (4) method was also qualified at the PGS-AND facility. The (b) (4)-BET method was performed using a (b) (4) and (b) (4)-BET method was performed using the (b) (4) the BET for Drug Product (DP), which also uses a (b) (4). The BNT162b2 DP is a single-stranded, 5'-capped mRNA that is translated into a protein. Lipids are later added to form lipid nanoparticles (LNP); (b) (4) the DP BET is performed.

Method description

(b) (4)

(b) (4)-BET Method Qualification for (b) (4)

(b) (4)

(b) (4)

BET Qualification for Drug Product

Pfizer qualified the (b) (4)-BET method for DP at the Pfizer Global Supply in Puurs, Belgium (PGS-Puurs) and Pfizer Global Supply in Kalamazoo, MI (PGS-KZO) facilities.

(b) (4)-BET Method Qualification for BNT162b2 mRNA DP:

The (b) (4)-BET is performed to detect or quantitate bacterial endotoxins present in test samples. (b) (4)

The original submission did not include qualification data from the PGS-Puurs and PGS-KZO laboratories. CBER sent an information request (IR) to Pfizer on July16, 2021, regarding their test for interfering factors at the PGS-Puurs and PGS-KZO facilities. Response to this IR was received on July 30, 2021 in amendment number 21.

Pfizer submitted supplemental verification reports performed on DP at each requested facility. These verification tests were performed with (b) (4)

(b) (4)

The current DP bacterial endotoxin test method involves sample preparation in which (b) (4) Pfizer performed a bacterial endotoxin (b) (4) test to demonstrate the (b) (4) . This (b) (4) test is described below:

(b) (4)

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(b) (4)

Conclusion
The endotoxin ((b) (4) ) test method was qualified appropriately and demonstrated to be suitable under the actual conditions of use. Pfizer has committed to (b) (4) and will submit the verification study results with a proposed updated lot release protocol template as a supplement to this file on or before December 6, 2021.

**14.** (b) (4) **Sterility Test**

Method description
The (b) (4) is used test DP sterility. (b) (4)

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(b) (4)

(b) (4)

(b) (4)

Conclusion: The (b) (4) Sterility Test method was validated in accordance with (b) (4) and was found to provide detection results equivalent (b) (4) sterility test method. The data provided show that the (b) (4) method is suitable for its intended use.