Australian Government
Department of Health and Aged Care
Therapeutic Goods Administration

Laboratories Branch

Type: Biotherapeutics\BPC\Methods	**Number:** Bio-BPC-Method-37 / **Version:** 2
Owner: s22	**Approver:** s22
Active: 13/10/2023	**Review:** 13/04/2025
Title: Pfizer - Lipid - Identification and Quantitation of ALC0159, Cholesterol, ALC0315 and DSPC in Pfizer Vaccine (Comirnaty)	

Identification and Quantitation of ALC0159, Cholesterol, ALC0315 and DSPC in Pfizer Vaccine (Comirnaty)

Purpose

The purpose of this method is the identification and quantitation of the lipids ALC-0159, cholesterol, ALC-0315 and DSPC in the Pfizer Comirnaty vaccine.

Scope

This method is suitable for Pfizer Comirnaty drug product (DP) samples tested in the Laboratories Branch at TGA.

Abbreviations

Term	Definition
µL	Microlitre
ALC-0159	2-[(polyethylene glycol)-2000]-N,N-ditetradecyclacetamide
ALC-0315	((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl) bis(2-hexyldecanoate)
CAD	Charged Aerosol Detector
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
LCMS	Liquid Chromatography Mass Spectrometry
RP-UHPLC	Reverse Phase Ultra-High Performance Liquid Chromatography
RT	Retention Time
SST	System suitability
STD	Standard

Method Reference

The method is based on the Pfizer method for Identification and Quantitation of Lipids in PF-07302048 by RP-HPLC with CAD detection by Pfizer (Document No. TM100010322, ver. 4.0 D20-3852581 and TM-01-9215A-00, ver. 1.0 D22-5558313).

Equipment, Materials and Reagents

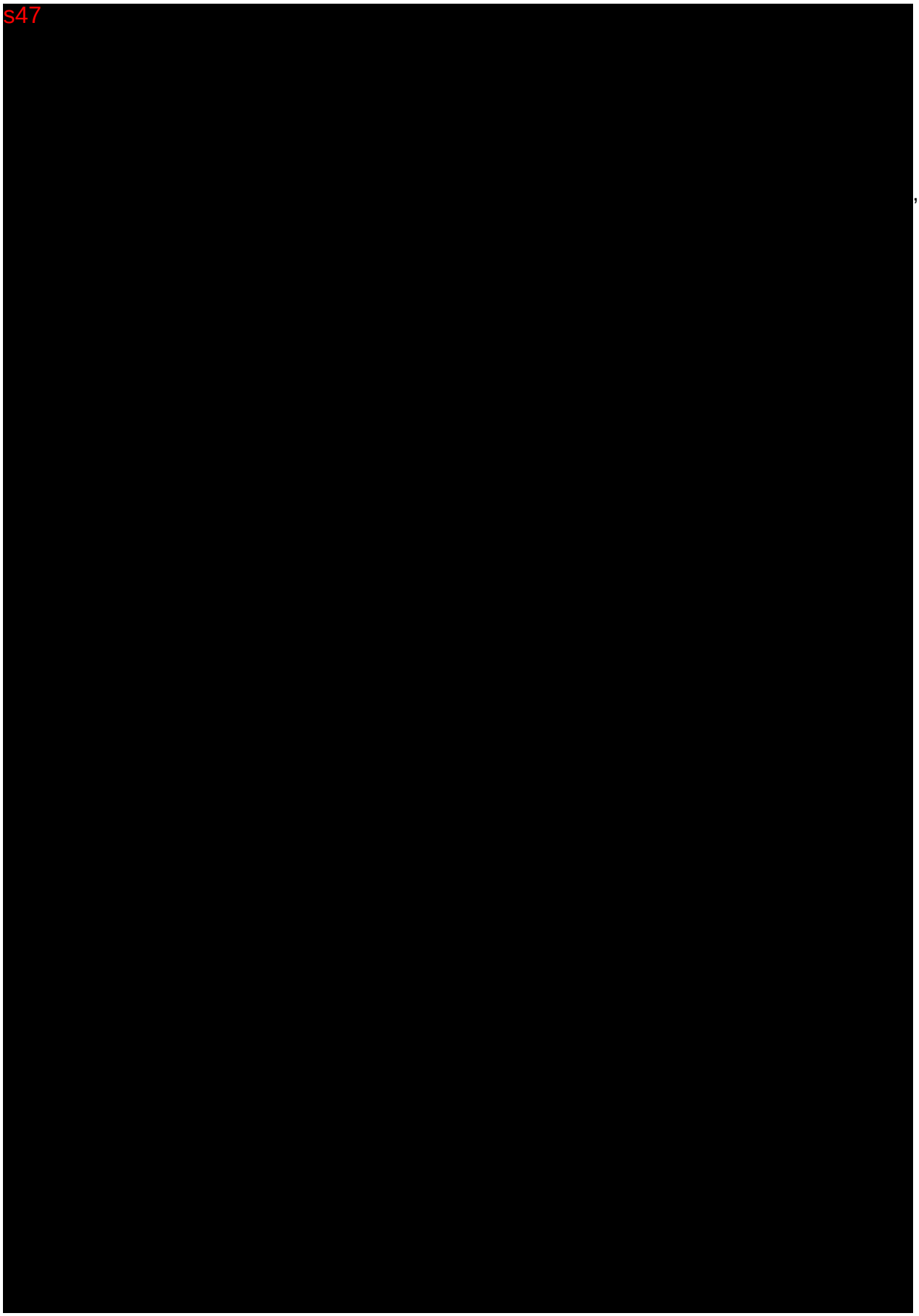
Equipment
Waters Acquity UPLC H-Class equipped with QSM to deliver gradient flow at 0.9 mL/min, SM-FTN, column oven, charged aerosol detector and eSAT/IN.
Waters Xselect CSH, C18 column, 4.6 mm x 150 mm, 3.5 µm (Waters part No. 186005270)
VanGuard Cartridge Holder (Waters part No. 186007949)
XSelect CSH VanGuard Cartridge, 130 A°, 3.5 µm x 5 mm (Waters part No. 186007813)
Analytical Balance, capable of reading to 0.1 mg
Sonication Bath
Eppendorf multipette
Vortex
Volumetric flasks
pH meter

Materials
Agilent Glass screw top, high recovery, HPLC vials (part No. 5183-2030) with Waters LCMS certified caps and pre-slit PTFE/Silicone septum (part No. 186005827).
Suitable combitips, recommend 1 and 2.5 mL (Catalogue number 0030089430 and 0030049448)

Reagents
Ultra-pure water, resistivity needs to be => 18.2 MΩ
Isopropanol (IPA), LCMS Grade
Methanol (MeOH), LCMS Grade
Acetonitrile (ACN), HPLC gradient Grade from Merck
Ammonium acetate, LCMS Grade
Acetic acid, LCMS Grade
Ammonia solution, LCMS Grade

Mobile phase preparation

s47



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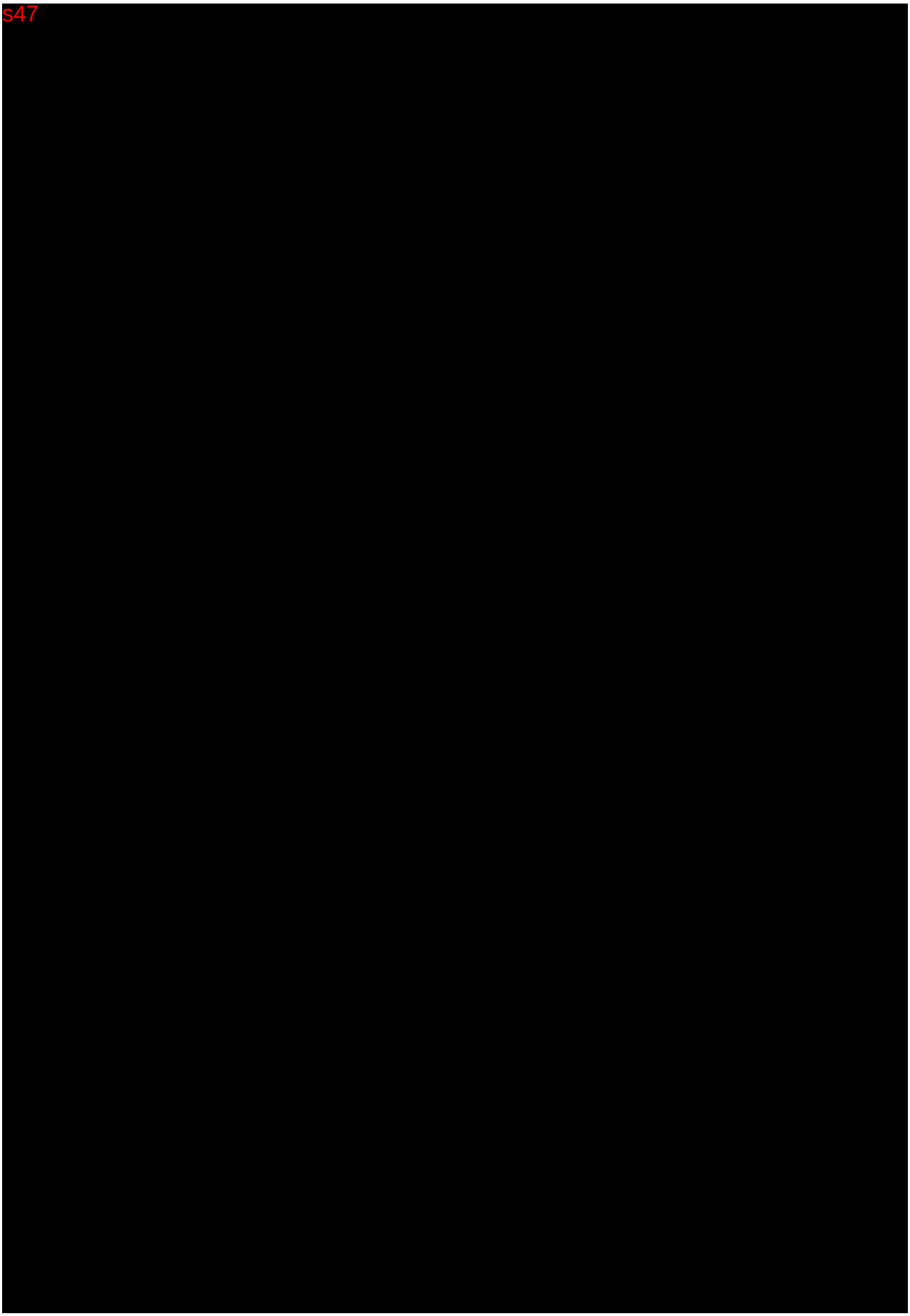

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Standard Preparation

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Assay control preparation:

s47 [redacted]

Preparation of Mixed lipid Calibration Standards

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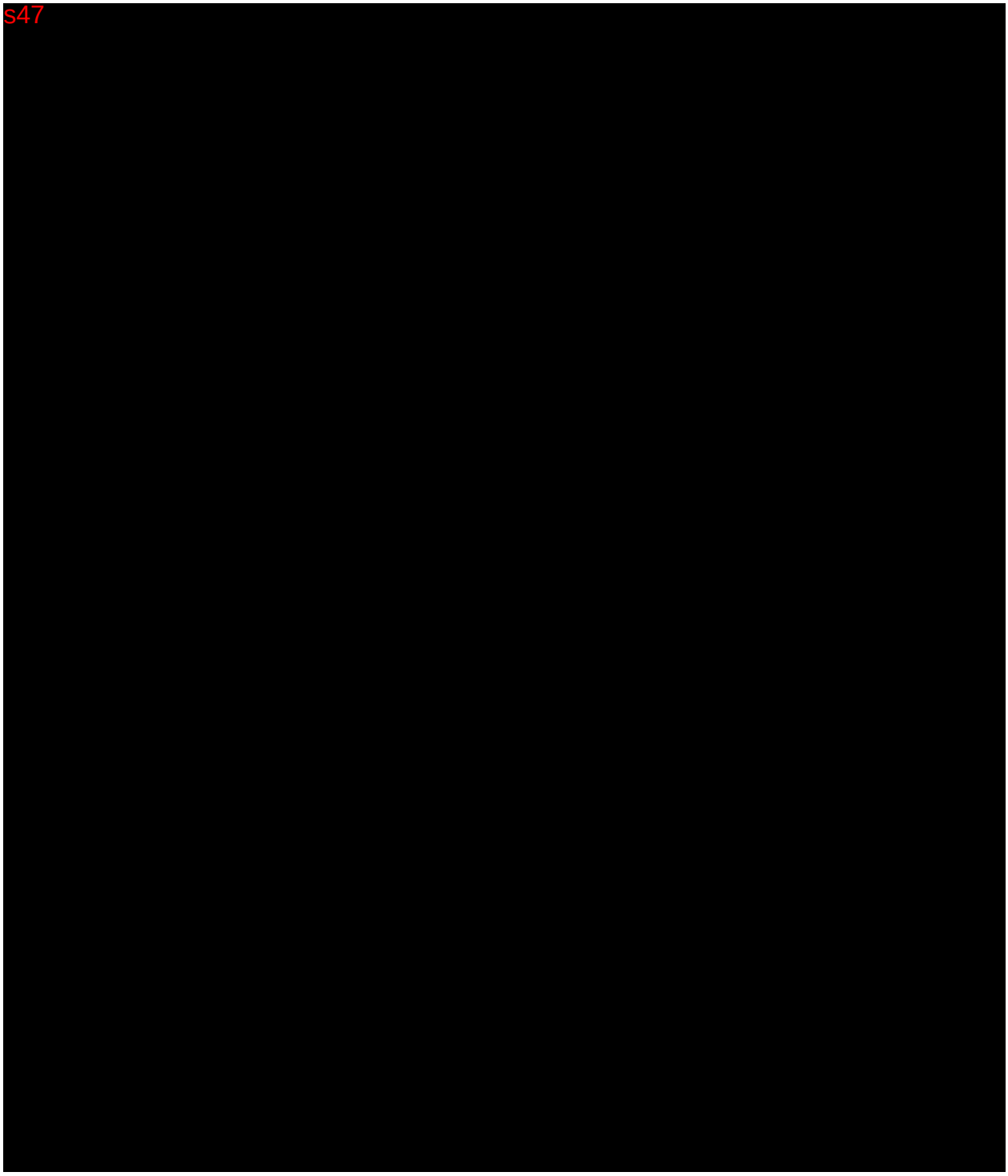

Preparation of drug product sample solutions

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Instrument setup:

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System suitability criteria

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Sample acceptance criteria

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Data processing and analysis:

s47 [redacted]

Reporting:

s47 [redacted]

Example chromatograms

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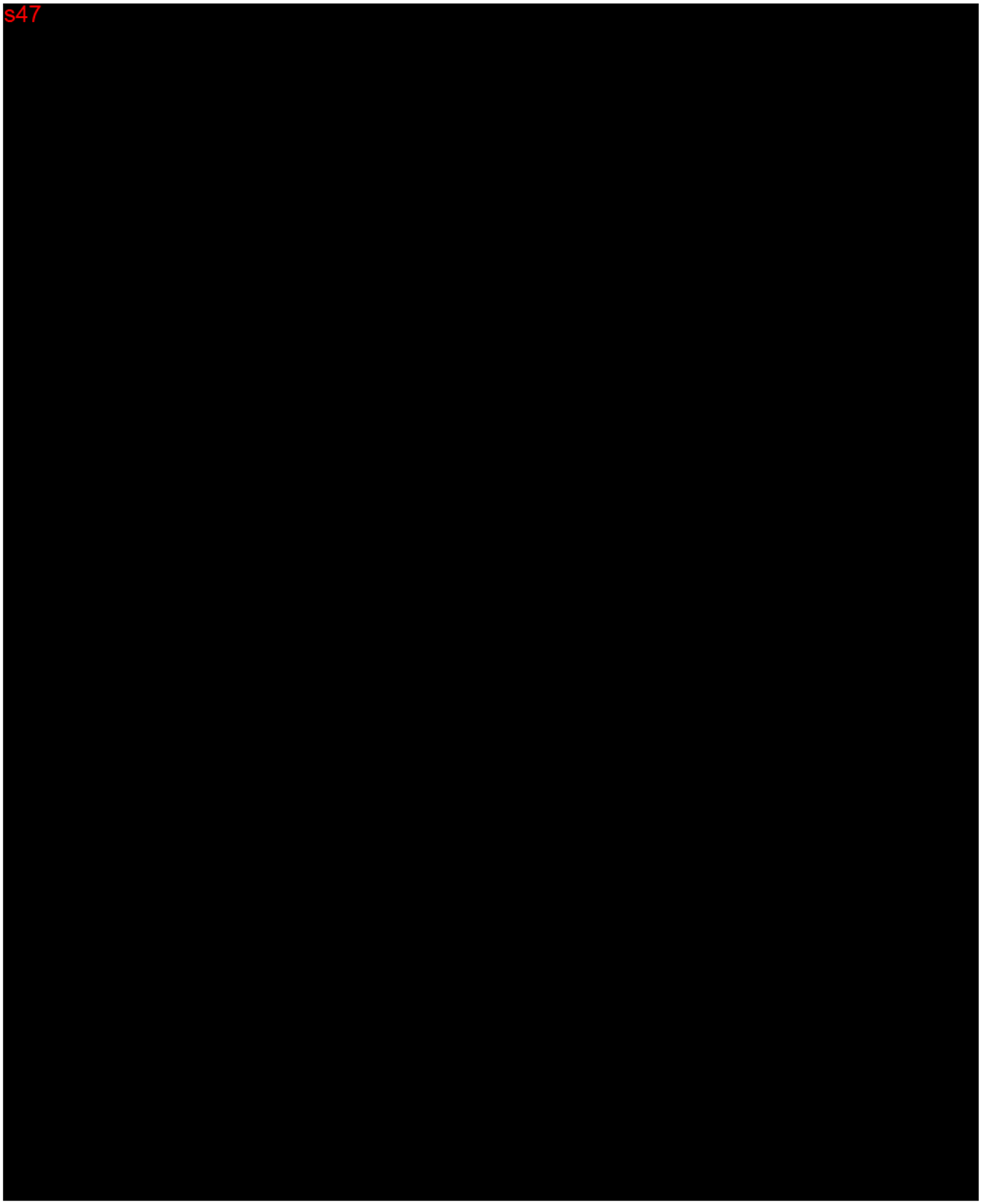

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: RNA integrity of mRNA drug substance and LNP-mRNA drug product samples by fragment analyzer	NUMBER: TM100010392	
		GDMS VER. 4.0	PAGE: 1 OF 24

1. PURPOSE

This laboratory method describes the procedure used to determine the percent integrity of messenger RNA (mRNA), specifically nucleoside-modified RNA (modRNA) for BNT162. This procedure also applies to RNAs that are isolated from BNT162 lipid nanoparticle (LNP) DPs by disruption of the nanoparticles.

2. SCOPE

This qualified procedure is applicable to drug substance and drug product release and stability samples.

3. RESPONSIBILITIES

s47 [redacted]

4. PRINCIPLE

s47 [redacted]

5. SAFETY

s47 [redacted]

6. DEFINITIONS

Table 1. Terms and Definitions	
TERM	**DEFINITION**
BSC	Biosafety cabinet
%CV	Percent coefficient of variation
DP	Drug product
DEPC	Diethylpyrocarbonate
DS	Drug substance
LNP	Lipid nanoparticle
mRNA	Messenger RNA
modRNA	Nucleoside-modified RNA (aka nmRNA)
N/A, NA	Not applicable
RNA	Ribonucleic acid

7. EQUIPMENT AND REAGENTS

Substitute materials, equipment or reagents can be used unless specified.

7.1 Equipment

Table 2. Equipment	
Item	**Manufacturer**
Fragment Analyzer Automated CE System	Agilent
Software: PROSize v3.0 or later	Agilent
48-Capillary Array, Short 33 cm	Agilent (A2300-4850-3355)
p10 µL pipette	Ranin
p20 µL pipette	Ranin
p200 µL pipette	Ranin
2-20 µL 12 channel pipette	Ranin
10-100 µL 12 channel pipette	Ranin
100-1000 µL 12 channel pipette	Ranin
ThermoMixer C	Eppendorf
SimpliAmp Thermal Cycler	Applied Biosystems (Thermo Fisher)
BioShake XP Thermoshaker	Q Instruments
Plate Centrifuge (mini benchtop single speed, or tabletop capable of 200 x g)	Axygen or Thermo
Biosafety cabinet (BSC) or PCR hood	LabConco. or AirClean Systems
Analytical Balance	Mettler Toledo
Vortex	VWR

7.2 Materials

Table 3. Materials		
Item	**Source**	**Catalog Number**
Pipette tips, sterile + filtered 0.1-20 µL 200 µL 300 µL 1000 µL	Biotix or substitute	 12-111-400 12-111-362 12-111-363 12-111-364
PCR-tubes, 1.5 mL, sterile	Eppendorf	022431081

Table 3. Materials		
Item	**Source**	**Catalog Number**
96-well PCR plates, sterile	Eppendorf	951020303
96 DeepWell Plates, 1 mL	Fisher Scientific	12566120
Adhesive PCR plate seal	Thermo	236366
Conical tube, 15 mL	Corning	430052
Conical tube, 50 mL	Corning	430290
Conical tube, 250 mL	Corning	430776
RNase Zap spray or wipes	Invitrogen	AM9782, or AM9786
70% alcohol wipes or spray(below)	Fisher Scientific	06-665-24
70% alcohol spray	Decon	8616
Germicidal Disposable Wipes	PDI	Q55172
PF-07305885 DS reference material (or other suitable material) is used as a control and subsequently referred to as a DS reference material in this test method.		
PF-07302048 DP reference material (or other suitable material) is used as a control and subsequently referred to as a DP reference material in this test method.		

7.3 Reagents

Table 4. Reagents		
Item	**Source**	**Catalog Number**
DEPC-treated water	Ambion	AM9916
Standard Sensitivity (SS) RNA kit: RNA separating gel Intercalating dye 5X 930 dsDNA inlet buffer RNA diluent marker RNA ladder 0.25x TE Rinse buffer BF-25 Blank solution	Agilent	DNF-471-1000 (or DNF-471-500) DNF-265-0500 DNF-600-U030 DNF-355-0300 DNF-369-0004 DNF-382-U020 DNF-497-0125 DNF-300-0008
5X Capillary Conditioning Solution	Agilent	DNF-475-0100
Capillary storage solution	Agilent	GP-400-0100
Triton X-100	Sigma	T8787-100ML
Ethanol	Fisher	BP2818
Purified Water	MilliQ, etc	N/A

Note: Kit options have identical components and differ in amount of kit reagents supplied. DNF-471-0500 is suitable for analysis of 500 plate wells, and DNF-471-1000 is suitable for analysis of 1000 plate wells.

8. PROCEDURE

s47 [redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: RNA integrity of mRNA drug substance and LNP-mRNA drug product samples by fragment analyzer	NUMBER: TM100010392	
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PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
Pfizer	TITLE: RNA integrity of mRNA drug substance and LNP-mRNA drug product samples by fragment analyzer	NUMBER: TM100010392	
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PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: RNA integrity of mRNA drug substance and LNP-mRNA drug product samples by fragment analyzer	NUMBER: TM100010392	
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PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: RNA integrity of mRNA drug substance and LNP-mRNA drug product samples by fragment analyzer	NUMBER: TM100010392	
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s47 [redacted]

9. INSTRUMENT SETUP

s47 [redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
Pfizer	TITLE: RNA integrity of mRNA drug substance and LNP-mRNA drug product samples by fragment analyzer	NUMBER: TM100010392	
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PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
Pfizer	TITLE: RNA integrity of mRNA drug substance and LNP-mRNA drug product samples by fragment analyzer	NUMBER: TM100010392	
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PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
Pfizer	TITLE: RNA integrity of mRNA drug substance and LNP-mRNA drug product samples by fragment analyzer	NUMBER: TM100010392	
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PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: RNA integrity of mRNA drug substance and LNP-mRNA drug product samples by fragment analyzer	NUMBER: TM100010392	
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PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
Pfizer	TITLE: RNA integrity of mRNA drug substance and LNP-mRNA drug product samples by fragment analyzer	NUMBER: TM100010392	
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PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
Pfizer	TITLE: RNA integrity of mRNA drug substance and LNP-mRNA drug product samples by fragment analyzer	NUMBER: TM100010392	
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PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: RNA integrity of mRNA drug substance and LNP-mRNA drug product samples by fragment analyzer	NUMBER: TM100010392	
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PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
Pfizer	TITLE: RNA integrity of mRNA drug substance and LNP-mRNA drug product samples by fragment analyzer	NUMBER: TM100010392	
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PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
Pfizer	TITLE: RNA integrity of mRNA drug substance and LNP-mRNA drug product samples by fragment analyzer	NUMBER: TM100010392	
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[redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
Pfizer	TITLE: RNA integrity of mRNA drug substance and LNP-mRNA drug product samples by fragment analyzer	NUMBER: TM100010392	
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PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: RNA integrity of mRNA drug substance and LNP-mRNA drug product samples by fragment analyzer	NUMBER: TM100010392	
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[redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
Pfizer	TITLE: RNA integrity of mRNA drug substance and LNP-mRNA drug product samples by fragment analyzer	NUMBER: TM100010392	
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PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
Pfizer	TITLE: RNA integrity of mRNA drug substance and LNP-mRNA drug product samples by fragment analyzer	NUMBER: TM100010392	
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[redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
Pfizer	TITLE: RNA integrity of mRNA drug substance and LNP-mRNA drug product samples by fragment analyzer	NUMBER: TM100010392	
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PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
Pfizer	TITLE: RNA integrity of mRNA drug substance and LNP-mRNA drug product samples by fragment analyzer	NUMBER: TM100010392	
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PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
Pfizer	TITLE: RNA integrity of mRNA drug substance and LNP-mRNA drug product samples by fragment analyzer	NUMBER: TM100010392	
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PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
Pfizer	TITLE: RNA integrity of mRNA drug substance and LNP-mRNA drug product samples by fragment analyzer	NUMBER: TM100010392	
		GDMS VER. 4.0	PAGE: 24 OF 24

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[redacted]

Document Approval Record

Document Name:	TM100010392
Document Title:	RNA Integrity of mRNA Drug Substance and LNP-mRNA Drug Product Samples by Fragment Analyzer

Signed By:	Date(GMT)	Signing Capacity
s22 [redacted]	25-Nov-2020 17:00:54	Author Approval
	25-Nov-2020 17:22:56	Manager Approval
	25-Nov-2020 22:17:51	Business Line Approver
	25-Nov-2020 23:27:58	Quality Assurance Approval

Method Verification Summary

Quantitation of Total and Percent Encapsulated RNA in PF-07302048 Lipid Nanoparticles by RiboGreen Fluorescence

The attached protocol (*Quantitation of Total and Percent Encapsulated RNA in PF-07302048 Lipid Nanoparticles by RiboGreen Fluorescence*) has been verified for use with Pfizer CovID-19 vaccine BNT162b2 with the following addenda:

- The Softmax Pro 6 experimental template file to be used for data collection and analysis is Pfizer BNT162b2 RiboGreen Fluorescence Assay Template.spr, and a copy is available at TRIM link: D21-2060144. This template file is also available on the Laboratories Branch I: drive and has been added to the Softmax Pro protocol library.

- This template contains instructions for entering LIMS numbers into the experiment file and generating .pdf reports.

Verification studies were performed in accordance with D18-11015470 GSM - 03 - Verification of Compendial or CPD methods – SOP.

Verification summary worksheet is available at: D21-2060341

Verification study data is available at: D21-2060760, D21-2060752

Analyst:

s22 [redacted], 14-Jan-2021 (signed electronically)

Reviewed:

s22 [redacted], 18-Jan-2021 (signed electronically)

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Quantification of Total and Percent Encapsulated RNA in PF-07302048 Lipid Nanoparticles by RiboGreen Fluorescence**	NUMBER: **TM100010402**	
		GDMS VER. **2.0**	PAGE. **1 OF 9**

1. PURPOSE

This method is used to quantify total RNA and percent RNA encapsulated in lipid nanoparticles in PF-07302048.

2. SCOPE

This method is qualified to test PF-07302048 drug product in-process, release, and stability samples.
s47 [redacted]

3. RESPONSIBILITIES

3.1 The analyst must complete all appropriate training prior to performing the method.

3.2 The analyst must follow this procedure as written and document all calculations appropriately.

3.3 The analyst must ensure all equipment is calibrated and capable of maintaining appropriate settings and conditions as specified in this method.

3.4 The analyst must report and properly document all deviations from the method procedure.

4. PRINCIPLE

s47 [redacted]

5. SAFETY

s47 [redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Quantification of Total and Percent Encapsulated RNA in PF-07302048 Lipid Nanoparticles by RiboGreen Fluorescence**	NUMBER: **TM100010402**	
		GDMS VER. **2.0**	PAGE. **2 OF 9**

s47 [redacted]

6. EQUIPMENT AND MATERIALS

s47 [redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Quantification of Total and Percent Encapsulated RNA in PF-07302048 Lipid Nanoparticles by RiboGreen Fluorescence**	NUMBER: **TM100010402**	
		GDMS VER. **2.0**	PAGE. **3 OF 9**

s47 [redacted]

7. PROCEDURE

s47 [redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Quantification of Total and Percent Encapsulated RNA in PF-07302048 Lipid Nanoparticles by RiboGreen Fluorescence**	NUMBER: **TM100010402**	
		GDMS VER. **2.0**	PAGE. **4 OF 9**

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PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Quantification of Total and Percent Encapsulated RNA in PF-07302048 Lipid Nanoparticles by RiboGreen Fluorescence**	NUMBER: **TM100010402**	
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PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Quantification of Total and Percent Encapsulated RNA in PF-07302048 Lipid Nanoparticles by RiboGreen Fluorescence**	NUMBER: **TM100010402**	
		GDMS VER. **2.0**	PAGE. **6 OF 9**

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PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Quantification of Total and Percent Encapsulated RNA in PF-07302048 Lipid Nanoparticles by RiboGreen Fluorescence**	NUMBER: **TM100010402**	
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PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Quantification of Total and Percent Encapsulated RNA in PF-07302048 Lipid Nanoparticles by RiboGreen Fluorescence**	NUMBER: **TM100010402**	
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PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Quantification of Total and Percent Encapsulated RNA in PF-07302048 Lipid Nanoparticles by RiboGreen Fluorescence**	NUMBER: **TM100010402**	
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Document Approval Record

Document Name:	TM100010402
Document Title:	Quantification of Total and Percent Encapsulated RNA in PF-07302048 Lipid Nanoparticles by RiboGreen Fluorescence

Signed By:	Date(GMT)	Signing Capacity
s22 [redacted]	07-Oct-2020 12:23:00	Business Line Approver
	07-Oct-2020 16:03:45	Author Approval
	14-Oct-2020 14:44:52	Business Line Approver
	16-Oct-2020 21:15:55	Quality Assurance Approval

Method Verification Summary

The attached protocol (*Identification of the mRNA in modRNA BNT162b2 (1525) Products using* s47 *PCR Assay*) has been verified for use with the following addenda:

- RNA extraction by use of QIAamp Viral RNA Mini Kit.
    - TRIM link to Mini Kit handbook: D21-2061208
    - Verification studies indicate the suitability of RNA extraction with the use of QIAamp Viral RNA Mini Kit, in lieu of the Pfizer manual RNA extraction SOP.
    - While an alternate means of extraction would potentially alter the yield and thus affect quantitative data, this assay relies on qualitative data only.
- s47 PCR performed using Applied Biosystems Quantstudio 3 s47 PCR system (QS3), in lieu of an Applied Biosystems 7500 s47 PCR system.
    - Performance of the method on the alternate has been verified in method verification studies, which met all system suitability criteria.
    - Thermal specifications of the QS3 system, as measured by NATA-accredited MTAS calibration procedure, Aug 2020, indicated temperature accuracy and uniformity performance comparable to the 7500 system specifications. (See MTAS calibration report, D20-3203138 and 7500 system specification sheet, D21-2061376). Measurements of QS3 thermal uniformity and accuracy showed better performance than the 7500 system specifications at the 50°C and 60°C set-points (critical temperatures for assay specificity). At the 95°C set-point, measurements of non-uniformity and inaccuracy in the QS3 were only slightly greater than for a 7500 system. This set-point is deemed less critical for method performance, if temperature overshoots do not lead to significant deterioration of polymerase enzyme activity. Such a loss of activity would give rise to false negative results in samples close to the limit of detection (late-amplifying, high Ct samples). Loss of polymerase activity was not observed in method verification studies, which depend upon the measurement of relatively early-amplifying, low Ct samples.
- Quantstudio experimental template for use in data collection and analysis is available in TRIM: D21-2061428

Verification studies were performed in accordance with D18-11015470 GSM - 03 - Verification of Compendial or CPD methods – SOP.

Verification summary sheet is available at: D21-2061501

Verification Data is available at: E21-207412, including assays D21-2061984, D21-2061990, and the study report at D21-2061970

Analyst:

s22 , 14-Jan-2021 (signed electronically)

Reviewed:

s22 16-Jan-2021 (signed electronically)

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Identification of the mRNA in modRNA BNT162b2 (1525) Products Using s47 PCR Assay**	NUMBER: **TM100010407**	
		GDMS VER. **3.0**	PAGE: **1 OF 31**

1. PURPOSE

This method describes a procedure for identification of the mRNA derived from the modRNA BNT162b2 (pST4-1525/ PF-07305883) products in Drug Substance (DS) and Drug Product (DP). s47 [redacted]

2. SCOPE

s47 [redacted]

3. RESPONSIBILITIES

3.1 The analyst must complete all appropriate training prior to performing the method.
3.2 The analyst must follow this procedure as written and document all calculations appropriately.
3.3 The analyst must ensure all equipment is calibrated and capable of maintaining appropriate settings and conditions as specified in this method
3.4 The analyst must report and properly document all deviations from the method procedure.
3.5 It is the responsibility of the analyst to report to management any problems encountered while performing this procedure.
3.6 It is the responsibility of the departments submitting samples to communicate sample delivery, and fill out a non-LIMS form, or provide LIMS sample information.

4. PRINCIPLE

s47 [redacted]

5. SAFETY

5.1 All laboratory work must be performed in compliance with Pfizer safety policies.
5.2 Some of the reagents used can cause skin, eye, and respiratory irritation. Wearing appropriate PPE during the handling of all reagents and working in a well-ventilated environment is recommended.
5.3 Avoid ingestion, inhalation and skin contact with samples and reagents.
5.4 Samples, and all materials that encounter the samples, will be treated as biohazardous waste.
5.5 All reagents, and materials that encounter the reagents, shall be treated as chemical waste.

6. DEFINITIONS

6.1 **PCR**– Polymerase Chain Reaction is an enzymatic reaction for amplifying specific sequences of nucleic acid in such quantities to allow for detection.

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PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Identification of the mRNA in modRNA BNT162b2 (1525) Products Using s47 PCR Assay**	NUMBER: **TM100010407**	
		GDMS VER. **3.0**	PAGE: **3 OF 31**

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[redacted]

6.13 **BSC** – Biological Safety Cabinet
6.14 **Cat#** – Catalogue number
6.15 **DS** – Drug Substance
6.16 **DP**– Drug Product
6.17 **eLN** – Electronic Laboratory Notebook
6.18 **IPA**– Isopropanol
6.19 **LIMS** – Laboratory Information Management System
6.20 **LNP** – Lipid Nanoparticle
6.21 **modRNA**– modified RNA
6.22 **mRNA** – Messenger Ribonucleic Acid
6.23 **MGBNFQ**– Minor Groove Binding Non-Fluorescent Quencher
6.24 **NTC** – Non-Template Control
6.25 **PCR** – Polymerase Chain Reaction
6.26 **PPE** – Personnel Protective Equipment
6.27 **RM** – Reference Material
6.28 [redacted] **PCR** – [redacted] Polymerase Chain Reaction
6.29 **TE Buffer** – Tris-EDTA Buffer
6.30 **TS** – Team Supply
6.31 **UNG** – Uracil-N-Glycosylase

7. EQUIPMENT AND REAGENTS

"Substitute", where indicated, means like-for-like replacement of materials or equipment from another source.

7.1 Equipment

7.1.1 Adjustable pipettes capable of delivering volumes between 5-1000 µL
7.1.2 Vortex mixer (VWR Mini Vortex 58816-121 or substitute)
7.1.3 Microcentrifuge (VWR Galaxy MiniStar C1413V-VWR230 or substitute)
7.1.4 Centrifuge with 500-1000 g capability, plate spinner or substitute
7.1.5 Biological Safety Cabinet Level 2 or PCR workstation as required

7.1.6 Freezer capable of maintaining -15 to -25°C and -60 to -90°C

7.1.7 Refrigerator 4-8°C

7.1.8 Ice Bucket/ Isotherm cooler blocks (as needed)

7.1.9 s47 PCR System (Applied Biosystems 7500 s47 PCR System or 7500 s47 s47 PCR System with SDS Software)

7.1.10 AutoMate Express Nucleic Acid Extraction System (Applied Biosystems)

7.1.11 Splash-free Support Base (Applied Biosystems, Cat# 4312063 or substitute)

7.2 Materials

7.2.1 Sterilized nuclease-free 1.5 mL microcentrifuge tubes (Ambion, Part#AM1240) or substitute

7.2.2 Nuclease free Amber tubes (blue, pink and brown, 500 µL) (Eppendorf, Cat# 022363638) or substitute

7.2.3 Nuclease free Amber tube 1.5 mL brown (Eppendorf, Cat# 022363514) or substitute

7.2.4 Suitable sterile and filtered pipette tips, RNase and DNase free, or substitute

7.2.5 MicroAmp™ Optical 96-Well Reaction Plate with/without Barcode & Optical Adhesive Films (Applied Biosystems, Cat# 4314320) or substitute

7.2.6 Adhesive Seal Applicator (Applied Biosystems, Cat# 4333183) or substitute

7.2.7 TE Buffer, 1X, Molecular Biology Grade (Promega, Cat# V6231) or substitute

7.2.8 s47 PCR Grade Water (Ambion, Cat# AM9935) or substitute

7.2.9 TaqPath™ 1-Step s47 PCR Master Mix, CG (Applied Biosystems, Cat# A15299) do not substitute

7.2.10 Water, nuclease-free (Invitrogen Cat# R0582) or substitute

7.2.11 RNase*Zap*™ RNase Decontamination wipe (Invitrogen, Cat# AM9786) or substitute

7.2.12 DNA AWAY™ Surface Decontaminant (Thermo Scientific, Cat# 7010) or substitute

7.2.13 70% EtOH (Sanihol, Cat# 8116) or substitute

7.2.14 70% IPA (Millipore Sigma, Cat#563935) or substitute

7.2.15 PrepSEQ™ Lysis Buffer (Applied Biosystems, Cat# A29825) do not substitute

7.2.16 PrepSEQ™ Express Nucleic Acid Extraction Kit (Applied Biosystems, Cat# 4466351) do not substitute

7.3 Reference Material

7.3.1 The Pfizer BTx Pharmaceutical Sciences PF-07305885 drug substance reference material (BNT162b2, modRNA1525 DS) is used as a Positive PCR control and subsequently referred

to as reference material in this test method. s47 [redacted]

7.3.2 The Pfizer BTx Pharmaceutical Sciences PF-07302048 drug product reference material (BNT162b2, modRNA1525 DP) is used as a Positive Extraction Control and subsequently referred to as DP reference material in this test method. s47 [redacted]

7.4 Preparation of the Positive PCR Control from DS Reference Material

s47 [redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Identification of the mRNA in modRNA BNT162b2 (1525) Products Using s47 PCR Assay**	NUMBER: **TM100010407**	
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[redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Identification of the mRNA in modRNA BNT162b2 (1525) Products Using s47 PCR Assay**	NUMBER: **TM100010407**	
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s47 [redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: Identification of the mRNA in modRNA BNT162b2 (1525) Products Using s47 PCR Assay	NUMBER: TM100010407	
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s47 [redacted]

8. PROCEDURE

s47 [redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Identification of the mRNA in modRNA BNT162b2 (1525) Products Using s47 PCR Assay**	NUMBER: **TM100010407**	
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PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Identification of the mRNA in modRNA BNT162b2 (1525) Products Using s47 PCR Assay**	NUMBER: **TM100010407**	
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s47 [redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Identification of the mRNA in modRNA BNT162b2 (1525) Products Using s47 PCR Assay**	NUMBER: **TM100010407**	
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[redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Identification of the mRNA in modRNA BNT162b2 (1525) Products Using s47 PCR Assay**	NUMBER: **TM100010407**	
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[redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Identification of the mRNA in modRNA BNT162b2 (1525) Products Using s47 PCR Assay**	NUMBER: **TM100010407**	
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[redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Identification of the mRNA in modRNA BNT162b2 (1525) Products Using s47 PCR Assay**	NUMBER: **TM100010407**	
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[redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Identification of the mRNA in modRNA BNT162b2 (1525) Products Using s47 PCR Assay**	NUMBER: **TM100010407**	
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[redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Identification of the mRNA in modRNA BNT162b2 (1525) Products Using s47 PCR Assay**	NUMBER: **TM100010407**	
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[redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Identification of the mRNA in modRNA BNT162b2 (1525) Products Using s47 PCR Assay**	NUMBER: **TM100010407**	
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s47 [redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Identification of the mRNA in modRNA BNT162b2 (1525) Products Using s47 PCR Assay**	NUMBER: **TM100010407**	
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[redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Identification of the mRNA in modRNA BNT162b2 (1525) Products Using s47 PCR Assay**	NUMBER: **TM100010407**	
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[redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Identification of the mRNA in modRNA BNT162b2 (1525) Products Using s47 -PCR Assay**	NUMBER: **TM100010407**	
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[redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Identification of the mRNA in modRNA BNT162b2 (1525) Products Using s47 -PCR Assay**	NUMBER: **TM100010407**	
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[redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Identification of the mRNA in modRNA BNT162b2 (1525) Products Using s47 PCR Assay**	NUMBER: **TM100010407**	
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[redacted]

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	TITLE: **Identification of the mRNA in modRNA BNT162b2 (1525) Products Using s47 PCR Assay**	NUMBER: **TM100010407**	
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[redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Identification of the mRNA in modRNA BNT162b2 (1525) Products Using s47 PCR Assay**	NUMBER: **TM100010407**	
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s47 [redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
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[redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: **Identification of the mRNA in modRNA BNT162b2 (1525) Products Using s47 PCR Assay**	NUMBER: **TM100010407**	
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s47 [redacted]

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[redacted]

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[redacted]

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[redacted]

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[redacted]

Document Approval Record

Document Name:	TM100010407
Document Title:	Identification of the RNA in modRNA BNT162b2 (1525) mRNA Using s47 PCR Assay

Signed By:	Date(GMT)	Signing Capacity
s22 [redacted]	04-Nov-2020 19:12:54	Author Approval
	05-Nov-2020 17:48:50	Business Line Approver
	05-Nov-2020 19:59:57	Quality Assurance Approval

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
	TITLE: Analytical Method for Size and Polydispersity Index Measurement in mRNA LNP Samples by Dynamic Light Scattering (DLS) Malvern Zetasizer	NUMBER: TM100010649	
		GDMS VER. < 1.0 >	PAGE: 1 OF 8

1. PURPOSE

The purpose of this document is to describe a dynamic light scattering (DLS) method for measurement of hydrodynamic size (diameter, nm) and polydispersity index (PDI) of COVID19 vaccine (COVID Vx) PF-07302048 lipid nanoparticle (LNPs) drug product (DP) and in-process samples.

2. SCOPE

This method applies to PF-07302048 (BNT-162, Covid-19 Vaccine) drug product and in-process DP samples tested in Biotherapeutics Pharmaceutical Sciences (BTxPS), Analytical Research and Development (ARD) and contract research organizations (CROs). The method is intended to use for the measurement of size (diameter, nm) and polydispersity index within the mRNA s47 [redacted] s47 [redacted]

3. RESPONSIBILITIES

3.1 It is the responsibility of the laboratory manager or designee to ensure that adequate safety assessments are performed on the materials required for this procedure prior to use.

3.2 It is the responsibility of the analyst to follow this procedure as written. All deviations from the method must be documented and properly reported.

3.3 Training on this method, on sample handling, and on all applicable equipment is performed per laboratory guidelines and is properly documented prior to analysis.

3.4 Document all activities appropriately in an Electronic Laboratory Notebook (or equivalent).

4. PRINCIPLE

DLS is a technique in which a beam of monochromatic laser light is directed through a suspension of particles in a solution and fluctuations in scattered light intensity are recorded, which is based on Brownian motion of the suspended particles. The scattering intensity fluctuation is analyzed through a time autocorrelation function by a digital correlator and the correlation function decay rate can be deconvoluted through a cumulant analysis for monomodal size distribution. Such analyses determine the translational diffusion coefficients D_t of the particles in solution, from which the hydrodynamic diameter d_h is calculated based on Stokes-Einstein relationship for spherical particles. Polydispersity index measures the extent of size distribution and can be determined through the cumulant analysis.

5. SAFETY

Avoid ingestion, inhalation and skin contact with samples and reagents by complying with personal protective equipment guidelines.

6. DEFINITIONS

6.1 ARD; Analytical Research and Development

6.2 BSC; Biosafety cabinet

6.3 DP; Drug product

6.4 eLN; Electronic lab notebook

6.5 LIMS; Laboratory information management system

6.6 LNP(s); Lipid Nanoparticle(s)

6.7 µL; Microliter

6.8 mg; Milligram

6.9 mL; Milliliter

6.10 mRNA; messenger ribonucleic acid

6.11 N/A; Not Applicable

6.12 nm; Nanometer

6.13 °C; Degrees Celsius

6.14 PCR; Polymerase chain reaction

6.14.1 P/N; Part number

6.14.2 RSD; Relative standard deviation

7. EQUIPMENT AND REAGENTS

Note: Equivalents may be used unless noted otherwise.

7.1 EQUIPMENT

7.1.1 Malvern Zetasizer Nano ZS. (**no equivalents**)

7.1.2 Calibrated pipettes to cover the volumes of 2 µl to 1000 µl.

7.1.3 Air Clean 600 PCR Workstation (AirClean® Systems)

7.1.4 Biosafety cabinet

7.2 MATERIALS

Note: Equivalents may be used unless noted otherwise.

7.2.1 Sterile syringe filters, Whatman Anotop 25, 0.1µm (Whatman; P/N 6809-2112).

7.2.2 All-plastic syringes (Fisher Scientific; P/N 14-817-29)

7.2.3 Aerosol barrier sterile pipette tips, 10 µL size (Rainin; P/N 30389175), 200 µL size (Rainin; P/N 30389188) and 1000 µL size (Rainin; P/N 30389165).

7.2.4 Eppendorf BIOPUR Safe-Lock Tubes 1.5 mL (Eppendorf; P/N 022600028)

7.2.5 Falcon™ 15mL Conical Centrifuge Tubes (Fisher scientific; P/N 14-959-53A)

7.2.6 150 mL Rapid-Flow Vacuum Filter Unit 0.1 µm aPES membrane (Nalgene P/N 565-0010)

7.2.7 Disposable, Malvern Panalytical Inc Cuvette (Fisher scientific; NC1572332 [1 mL])

7.3 REAGENTS

Note: Equivalents may be used unless noted otherwise.

7.3.1 Nanosphere size standard (Thermo Fisher Scientific; P/N 3150A) (**no equivalents**).

7.3.2 Gibco™ DPBS, no calcium, no magnesium, 1X Solution (Fisher Scientific; P/N Gibco™ 14190144)

8. PROCEDURE -PREPARATION OF PBS, STANDARD AND SAMPLES

s47 [redacted]

PHARMACEUTICAL SCIENCES		ANALYTICAL METHOD	
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9. MEASUREMENT-PROCEDURE

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s47 [redacted]

10. ANALYSIS

s47 [redacted]

11. SYSTEM SUITABILITY CRITERIA

s47 [redacted]

12. ASSAY ACCEPTANCE CRITERIA

s47 [redacted]

13. REPORTING

s47 [redacted]

14. REFERENCES

s47 [redacted]

15. LIST OF ATTACHMENTS

N/A

16. REVISION HISTORY

s47 [redacted]

-END OF PROCEDURE-

Document Approval Record

Document Name:	TM100010649
Document Title:	Analytical Method for Size and Polydispersity Measurement in mRNA LNP Samples by Dynamic Light Scattering (DLS) Malvern Zetasizer

Signed By:	Date(GMT)	Signing Capacity
s22 [redacted]	03-Sep-2020 21:11:24	Scientific Review
	03-Sep-2020 21:13:45	Author Approval
	03-Sep-2020 22:00:54	Business Line Approver
	04-Sep-2020 01:17:35	Manager Approval
	04-Sep-2020 12:44:47	Quality Assurance Approval

Australian Government
Department of Health and Aged Care
Therapeutic Goods Administration

Laboratories Branch

Type: Biotherapeutics\BEE\Methods	**Number:** Bio-BEE-Method-7 / **Version:** 1
Owner: s22 [redacted]	**Approver:** s22 [redacted]
Active: 2/05/2022	**Review:** 7/02/2025
Title: Performing an Endotoxin Assay Kinetic or rFC	

Performing an Endotoxin Assay – Kinetic or rFC

Purpose

The purpose of this method is to describe the procedures to be followed when performing an Endotoxin Assay within Biotherapeutics

Standard Operating Procedure (SOP)

For full details of the endotoxin testing refer to Bio-BEE-SOP-28 – Endotoxin Testing - Kinetic and rFC.

Equipment and Reagents

All equipment used in the Endotoxin assays must be free of detectable endotoxin and of interfering effects for the assay. Careful technique is also required to avoid endotoxin contamination. Refer to Bio-BEE-SOP-28 for the equipment and materials required to perform the endotoxin assays. Bio-BEE-Method-6 describes the preparation of the reagents used.

Assay Set-Up

Move to the lab bench to begin setting up the assay.

Ensure that the correct (pyrogen-free) tips are used. Obtain the required pipettes, reagent reservoir (keep in bag), dilution tubes or bottles, reaction plate, tip discard, sharps bin etc.

- Remove the kit reagents required for this assay from the designated storage and allow to equilibrate to room temperature before use.
- If the CSE to be used has not yet been reconstituted, follow the procedure as set out in Bio-BEE-Method-5. CSE dilutions can be dispensed to the plate as they are prepared to save mixing time
- Open the seal around the reaction plate and retain the plastic base of the seal. This can be used as a non-pyrogenic surface for temporary storage of the rubber stoppers for the LRW and lysate vials that need to remain pyrogen free.
- Keep the lid on the plate and if desired, mark the assay on the lid of the plate as per the Template Plate Layout.
- Carefully open the aluminium seal on the LRW vial and discard metal into a sharps bin
- Load CSE onto the plate as detailed in the Method being performed.

Preparation of Sample Dilutions

When performing an assay to test samples you will also need to set up the appropriate tubes for predilution and testing of samples before proceeding.

Sample dilutions are prepared in pyrogen free tubes or bottles. Diluting samples to the routine test dilution as recorded in Bio-BEE-Form-42.

- Retrieve required samples from designated storage to equilibrate to room temperature before use
- Rack and label the appropriate number of dilution tubes required for each sample, as set out in Bio-BEE-Form-42. If using pyrogen free test tubes, remove from the bottom of the foil packaging ensuring you do not introduce contamination. If using the black lid screw-top bottles or pre-used tubes, ensure that they have been depyrogenated.
- Open the LRW vial, resting the stopper upside down in the pyrogen-free base of the plate seal.
- Dispense LRW into tubes/bottles as set out in Bio-BEE-Form-42
- Ensure that the appropriate PPC spikes have been added to wells as per the plate layout
- Continue to prepare samples as outlined in Bio-BEE-Method-2 or Bio-BEE-Method-3

Starting the Assay

- Once all samples have been added to the plate, it is then ready for the reaction.
- Move on to preparing the software as detailed in Bio-BEE-Method-4, to the point of Pre-warming the plate.
- Leave the lid of the plate on, place the plate into the reader and click OK, this starts the 10-minute warm-up
- Prepare the required lysate vial/s or rFC reagent as detailed in Bio-BEE-Method-6 and immediately before use, pour the contents from the vial/s into the reagent reservoir

After reconstitution of Lysate or mixing of rFC reagent

It is very important to be quick and precise when adding the lysate or rFC reagent to the plate, and to avoid bubbles and frothing. The lysate or rFC reagent can be dispensed using normal or “reverse” pipetting. Most bubbles will disappear after about 10 seconds.

- Move the tip-discard close to the plate reader and ensure the 8 channel pipettor (and the single channel pipettor) is set at 100 µl
- Move the reagent reservoir with lysate or rFC reagent next to the plate reader and position comfortably
- **Note:** When adding the last column of lysate or rFC reagent to the plate there is usually not sufficient liquid in the reservoir to continue using 6 or 8 channels, so it is best to use 2 or 4 tips close to the end

Add lysate or rFC reagent to the plate - 2 or more sets of 8 wells, 1 or 2 lysate vials. Or > 2.8 mL rFC reagent (IQ assay - 4 sets of either 6 wells (KLAL) **or** 5 wells (*rFC*), 1 lysate vial or 2.6 mL rFC reagent

- Remove the plate from the reader (refer to Bio-BEE-Method-4) and perform the following on the bench.
- Engage 8 tips onto the multichannel pipettor and perform pre-wet

- Using the same set of tips and touching just the very top left-hand sides of the wells (or nothing at all), pipette 100 µl into the first column of the plate carefully, and as quickly as possible.
- Using the same set of tips, repeat with the subsequent columns of the plate. If the number of samples tested means that most or all the lysate or rFC reagent will be used, the final column should be pipetted using two tips at a time
- Return the plate to the reader, using the software (refer to Bio-BEE-Method-4)
- Click OK to start the run. **Do not open drawer**

Assay Acceptance Criteria

The standard curve for each assay must meet the appropriate parameters, as detailed in BEE-SOP-28, for the assay to be considered valid.

Recording Results

- Prior to exporting, the Operator should electronically sign the report and record any deviations from the method during the e-signature procedure
- Have another operator record any explanation of unexpected outcomes and sign electronically as a "Reviewer" (or trim workflow where appropriate)
- Transcribe results to Assay Worksheet and Sample Result Sheet/s, i.e. % CV's, acceptance criteria parameters, EU/ml, and % PPC Recovery

The results are stored as part of the assay in the WinKQCL software. Exported versions are stored in Endotoxin Testing Results folders.

Associated Documents

- Bio-BEE-SOP-28 – Endotoxin Testing - Kinetic and rFC
- Bio-BEE-Method-1 – Initial and Operator Qualification Endotoxin Assay
- Bio-BEE-Method-2 – Endotoxin Routine Assay
- Bio-BEE-Method-3 – Endotoxin Bexsero Assay
- Bio-BEE-Method-4 – Software and Equipment Use
- Bio-BEE-Method-5 – CSE Preparation
- Bio-BEE-Method-6 – Reagent Preparation for Endotoxin
- Bio-BEE-Method-8 – Product Specific Details for Endotoxin
- Bio-BEE-Form-34 – Kinetic LAL Bexsero Assay Worksheet
- Bio-BEE-Form-35 – Media Preparation Water Worksheet
- Bio-BEE-Form-36 – Initial and Operator Qualification Worksheet
- Bio-BEE-Form-37 – CSE Preparation
- Bio-BEE-Form-39 – Endotoxin Routine Assay Worksheet
- Bio-BEE-Form-42 – Endotoxin Routine Assay Sample Results Sheet

Australian Government
Department of Health and Aged Care
Therapeutic Goods Administration

Laboratories Branch

Type: Biotherapeutics\BEE\SOP	**Number:** Bio-BEE-SOP-28 / **Version:** 3
Owner: s22	**Approver:** s22
Active: 15/08/2023	**Review:** 15/02/2025
Title: Bacterial Endotoxin Testing - Kinetic LAL and rFC	

Bacterial Endotoxin Testing - Kinetic LAL and rFC

Purpose

The purpose of this document is to describe the Standard Operating Procedure used for testing the bacterial endotoxin content of vaccines and other parenterally administered medicines with the kinetic chromogenic LAL (KLAL) and recombinant factor C (*rFC*) tests. The procedures contained within this document are performed in accordance with the harmonised methods of Ph. Eur. 2.6.14 and USP <85> (KLAL) and Ph. Eur. 2.6.32 (*rFC*). The results obtained using this procedure, including any deviations, will be documented in the appropriate worksheets.

Scope

The scope of this Standard Operating Procedure is limited to the Biotherapeutics Section of the Laboratories Branch (LB), TGA. The procedures within this manual are used to measure the endotoxin content of vaccines (especially those targeted against covid, typhoid and influenza) and can also be used to monitor the endotoxin content of many other therapeutic goods. Any changes to the procedure would need to be assessed to determine whether a re-validation is necessary.

Responsibility

Only valid operators from the Biotherapeutics Section, LB are to carry out this procedure.

The maintenance of this document is the responsibility of the Senior Scientist. Approval of any changes can be performed by the Biotherapeutics Director.

Before performing this procedure for routine assays, operators must be officially trained according to the Bacterial Endotoxin training sheets. Operators are required to pass an initial qualification (IQ) assay for the KLAL or *rFC* methods before performing further testing.

Background

A pyrogen is a substance that causes a rise in body temperature. The presence of pyrogens in drugs and devices that come into contact with a large volume of body fluids can allow rapid distribution of pyrogens and potentially cause a febrile reaction. These products are required to be tested for the presence of pyrogens. The most common pyrogens are bacterial endotoxins, but numerous other pyrogens exist. Bacterial endotoxins are not inactivated by normal sterilisation procedures, remaining active in or on the surfaces of therapeutic goods after bacterial cell death.

Pyrogen testing was initially carried out in rabbits. This test consists of measuring the rise in body temperature evoked in rabbits by the intravenous injection of a sterile solution of the substance to be

examined. This procedure non-specifically tests for all pyrogens and requires the use of animals. It is time-consuming, expensive, and on ethical grounds is actively discouraged in favour of in vitro bacterial endotoxin testing. Rabbit pyrogen testing has largely been superseded by bacterial endotoxin testing, but remains a compendial test, as some products do cause interference with endotoxin testing.

Endotoxins from Gram-negative bacterial cell walls (LPS) are the most common cause of the toxic reactions attributed to contamination of pharmaceutical products with pyrogens; their pyrogenic activity is much higher than that of most other pyrogenic substances.

For KLAL, the test for bacterial endotoxins uses a lysate of amoebocytes from the blood of the horseshoe crab, *Limulus polyphemus*. Gram-negative bacterial endotoxin triggers an enzyme cascade leading to the activation of a proenzyme in the Limulus Amoebocyte Lysate (LAL).

The addition of a solution containing endotoxins to a solution of the lysate produces turbidity, a chromogenic reaction or gelation of the mixture depending on the type of lysate preparation. The rate of reaction depends on the concentration of endotoxin, the pH and the temperature. The kinetic chromogenic lysate used in this assay contains certain divalent cations, proenzymes and a peptide substrate linked to the coloured product p-nitroaniline which is read at 405 nm. The presence of endotoxins in a product may be masked by factors interfering with these enzyme dependent reactions.

For *rFC*, the test for bacterial endotoxins uses recombinant Factor C, an endotoxin-sensitive protein. rFC is used in combination with a fluorogenic substrate. Factor C, the first component in the cascade, is a protease zymogen that is activated by endotoxin binding. Studies have demonstrated the ability of Factor C to selectively recognise endotoxin and activate the protease cascade. To create an endotoxin-specific assay, Factor C has been purified and cloned. When activated by endotoxin binding, rFC acts upon the fluorogenic substrate in the assay mixture to produce a fluorescent signal in proportion to the endotoxin concentration in the sample. The fluorescence is measured at time zero and after a one-hour incubation at 37°C, using excitation/emission wavelengths of 380/440 nm. The difference between the one-hour reading and the time zero reading (ΔRFU) is corrected for blank ΔRFU fluorescence. The log net fluorescence is proportional to the log endotoxin concentration.

Both tests are carried out in a manner that avoids microbial contamination using endotoxin free reagents and materials.

Prior to routine testing, the product to be tested must have been shown not to interfere with the assay. A test for interfering factors is conducted on 3 batches of the product (wherever possible) to determine the routine dilution at which no interference is observed. Please read the Bacterial Endotoxin Testing Manual (ie this SOP and the associated methods) for more information regarding the validation/qualification of the method for use with different products.

References

- European Pharmacopoeia 2.6.14 - Bacterial Endotoxins
- European Pharmacopoeia 2.6.32 - Bacterial Endotoxins using Recombinant Factor C
- United States Pharmacopoeia <85> - Bacterial Endotoxin Test
- Documentation from kinetic chromogenic kit (Lonza Limulus Amoebocyte Lysate (LAL) Kinetic-QCL)
- Documentation from Pyrosperse™ Dispersing Agent (Lonza Cat# N188)
- Documentation from recombinant factor C kit (currently Lonza Pyrogene™ Endotoxin Detection Assay)

- FDA Guidance for Industry – Pyrogen and Endotoxins Testing: Questions and Answers
- Current Risk Assessment for bacterial endotoxin testing procedures

WHS Requirements

Risk Assessment

Review the Risk assessments before performing the assays. the Laboratory Operations Manual details the WHS aspects of our work. Appropriate PPE must be worn when performing this procedure. There are no extraordinary requirements specific for this assay. A link to the risk assessment tool can be found in the TGA Laboratories Quality Manual (Risk Assessment Program). From the "New and edit" drop down menu select Open/edit assessment. At the next page scroll down to find the appropriate assessment. The risk assessment for this procedure (Endotoxin Manual) is 91215272004.

Safety Data Sheets (SDS)

Relevant information regarding the safety data sheet for the endotoxin test kits can be accessed via ChemWatch GoldFFX (https://jr.chemwatch.net/chemwatch.web/account/autologinbyip/). Relevant details regarding login and use of GoldFFX can be found in the Quality System - System Management - Work Health & Safety (WHS)

Training

Only suitably trained, staff should perform the assays described in this SOP. Before performing an initial qualification (IQ) assay, operators must be officially trained up to the relevant point according to the appropriate training sheet. Staff members to be trained in the assay should read the entire Bacterial Endotoxin Testing Manual before proceeding.

Waste disposal procedures

The policy for disposal of assay waste is outlined at: HPRG Intranet - Health & Safety - Work health and safety - Laboratory safety - Personal conduct and housekeeping within the laboratory. Local procedures for the disposal of waste can be found in LB02-36 - General Laboratory Safety.

Limulus Amoebocyte Lysate (LAL)

This is a freeze-dried preparation that is based on the blood cells of horseshoe crabs. For this assay the lysate incorporates a substrate that is cleaved by the enzyme cascade to release a chromogenic product.

The LAL Reagent, or lysate, should be reconstituted according to the manufacturer's instructions. Currently, using Lonza's KQCL kit, this involves reconstitution with 2.6 ml of LAL Reagent Water (LRW). This should only be done immediately before it is required for use because once reconstituted, background cleavage of the substrate can occur, resulting in very low levels of colour formation. Prolonged exposure to air, light and potential endotoxin contamination should all be avoided. The reconstituted LAL reagent is stable if stored protected from light at 2-8°C for 8 hours. Alternatively, it can be stored at or below -10°C for up to 14 days and thawed only once.

Care must be taken when reconstituting lysate to avoid frothing. The vial should be swirled gently or rolled. Shaking too vigorously can lead to the proteins denaturing, reducing enzyme activity and also causes bubbles in the reaction plate that will not allow the kinetic assay to be analysed correctly by the plate reader.

Pyrosperse™ Dispersing Agent (used in conjunction with KQCL kit - specifically validated products only)

Is intended for use with the KQCL assays to assist in the qualitative or quantitative detection of bacterial endotoxin. Pyrosperse™ is one of a group of metallo-modified polyanionic dispersants which has proven useful as a sample modifying agent for certain types of products showing inhibition in the LAL assay. In those products for which endotoxin binding is the suspected source of inhibition, the use of Pyrosperse™ should be considered. To date, Pyrosperse™ has been found useful in LAL endotoxin detection when used with the following products: Human Serum Albumin, 5% and 25%; Plasma Protein Fraction; Electrolyte solutions; Antihemophilic Factor; and Lipid emulsions. Additional product applications may exist.

rFC Working Reagent

Is made by mixing the fluorogenic substrate, rFC assay buffer and the rFC enzyme solution at a 5:4:1 ratio. Refrigerate any unused reagents after opening. Previously opened vials remain effective throughout the lifetime of the reconstituted endotoxin if returned to refrigeration. Note: The order of mixing should be as prescribed above - add enzyme last to the buffered substrate. Mix thoroughly but gently after each addition into the reagent reservoir. Do not vortex the working reagent mix. Once prepared, the working reagent cannot be stored. The working reagent is prepared at the last step – usually whilst the loaded plate is pre-incubating.

To ensure enough working reagent is prepared for the assay and excess is minimal, determine the number of wells requiring working reagent and add 4 additional wells. Calculate the appropriate amount for each component to make the working reagent. Equilibrate reagents to room temperature before mixing. A table showing volumes of each component can be found in the Pyrogene™ rFC booklet (trim). An excerpt from the booklet is shown below.

1 - Volume (uL) of Reagent Required per Total Number of Wells

Total Number of Wells	Fluorogenic Substrate	rFC Assay Buffer	rFC Enzyme Solution	Total Volume
12	800	640	160	1600
24	1400	1120	280	2800
36	2000	1600	400	4000
42	2300	1840	460	4600
48	2600	2080	520	5200
54	2900	2320	580	5800

Control Standard Endotoxin (CSE)

The Control Standard Endotoxin CSE should be reconstituted according to the manufacturer's instructions. Currently, using Lonza's KQCL or Pyrogene™ kit, this involves reconstitution with LAL Reagent Water (LRW). The endotoxin concentration is specific to each batch of CSE when matched to a specific lysate or rFC reagent lot and calibrated against the international Reference Standard Endotoxin (RSE). The volume is different for each batch of CSE so that the vial is made up to the correct endotoxin concentration of 50 EU/ml (KLAL) or 20 EU/ml (*rFC*). The correct volume to be added is available on the Certificate of Analysis from the Lonza website.

The CSE is lipopolysaccharide purified from bacterial cell walls. It is not easily maintained in solution and will readily adhere to the vial and to itself. For this reason, all standard solutions must be mixed very thoroughly. The reconstituted vial is shaken vigorously on a vortex for at least 15 minutes before use. Once reconstituted the CSE must be stored at 2-8°C and used within 4 weeks (28 days). When

using a vial that has been reconstituted previously, the vial must be equilibrated to room temperature and vortexed for 15 minutes prior to each use.

Standard Curve and Positive Product Controls

The CSE standard curve for **most** endotoxin testing on the WinKQCL system consists of 5 (KLAL) or 4 (*rFC*) solutions, each being a dilution of the previous. Due to the purity and polar nature of the CSE, the standard vials and tubes should be vortexed on high for 1 minute before making any dilution and before taking aliquots for the assay plate. The concentrations are **50**, 5.0, 0.5, 0.05 & 0.005 EU/ml (KLAL) and 5, 0.5, 0.05 & 0.005 EU/ml (*rFC*) and the Positive Product Control (PPC) spikes are usually set in the middle of the curve at 0.5 EU/ml.

The PPC spike is therefore usually a 10 µl aliquot of the 5 EU/ml standard pipetted directly into the appropriate empty wells of the assay plate. A 100 µl aliquot of each sample is then placed into the appropriate 4 wells of the assay plate for the test and PPC.

For a routine assay, the standards, samples and controls are tested in duplicate. A 100 µl aliquot of each standard and the negative control is placed into the appropriate wells of the assay plate. The use of a tube or bottle to contain the negative control (LRW) prior to dispensing to the plate, while strictly correct, is not usual practice for endotoxin testing. This also applies to the 50 EU/ml standard for KLAL. The appropriately diluted samples are then added to the plate, again in 100 µl volumes. The assay is begun with the addition of lysate (kinetic) or rFC reagent (*rFC*) while incubating the plate at 37°C.

Some products are tested using different sets of standards and/or different endotoxin concentrations for the PPC. For example, Bexsero (KLAL) uses a 3-point standard curve and a PPC concentration approximating 1.0 EU/ml. Typhim Vi and Vivaxim are also validated to use these assay parameters.

Considerations before performing an assay

Bacterial Endotoxin Limit

This is the maximum allowable amount of bacterial endotoxin in a product, measured in either EU/ml or EU/mg. Some products have established endotoxin limits set in pharmacopoeial monographs. For other products, calculation of the limit can be performed using the following formula:

$$\text{Endotoxin Limit} = K / M$$

Where: K is the maximum allowable pyrogenic dose (EU/kg/h)

M is the maximum recommended dose of the product (amount/kg/h)

Companies sometimes set endotoxin limits stricter than the pharmacopoeial or calculated values.

Maximum Valid Dilution (MVD)

The MVD is the value, calculated using the endotoxin limit of the product and the sensitivity of the assay, that the product can legitimately be diluted to for testing.

$$MVD = \frac{\text{Endotoxin Limit} \times \text{Product Concentration}}{\text{Lysate or rFC sensitivity } (\lambda)}$$

pH Values

The reactions that drive this assay are enzyme based and do not work as effectively outside a pH range of 6 - 8. rFC reagent is a solution that contains rFC buffer and lysate is lyophilised (and therefore reconstituted) in a formulation that buffers the pH effects of most products. If there is a chance that the pH of the product under investigation may cause assay interference due to pH, then appropriate dilutions of the product added to rFC reagent or lysate should be tested for pH.

Analyst and Lysate Qualification

An initial qualification (IQ) assay must be performed on each new batch of lysate or rFC reagent. All analysts are required to perform this IQ assay before doing routine assays. Analysts who have not maintained their competency by performing at least one valid routine assay within a 12-month period are obliged to perform this IQ assay before doing routine assays. The assay consists of constructing the normal standard curve and testing it in quadruplicate.

The outcome of the assay depends heavily upon the timing and technique of adding the lysate or the rFC reagent to the reaction plate. This is especially evident when performing an IQ assay due to the quadruplicate nature of the assay.

When performing an IQ assay, follow the procedures as described in Bio-BEE-Method-1, with reference to Bio-BEE-SOP-28 – Endotoxin Testing - Kinetic LAL and rFC and worksheet (Bio-BEE-Form-36 – Initial and Operator Qualification Worksheet).

Appropriate acceptance criteria must be met for the lysate or rFC and operator to be qualified.

Preparatory Testing

Due to the biological nature of the assay reaction, it is not known whether a new product will interfere with the lysate or rFC reagent. To ensure both precision and validity of the test, preparatory testing is often performed on new products. A test for interfering factors, or inhibition/enhancement test should first be conducted.

Product interference can generally be overcome by appropriate dilution of the product. Therefore, the bacterial endotoxin test must be qualified for each different product by demonstrating the absence of significant inhibition or enhancement by that product, on the test method at certain dilutions.

An initial screening assay can be done on one batch of new product at a range of different dilutions within the MVD to establish an appropriate dilution at which to perform the test for interfering factors.

Obviously, the endotoxin limit for the product and factors such as pH also need to be considered when doing this screen.

If products interact directly with the lysate or rFC constituents or otherwise interfere at the MVD, then another lysate or rFC manufacturer (Charles River, Associates of Cape Cod) could be trialled, or the gel clot test could be used.

Once an appropriate dilution is determined, a full test for interfering factors is conducted. This is a test on 3 batches of the product (sometimes this is not logistically possible), to confirm initial screen results and to establish a routine test concentration, which neither inhibits nor enhances the lysate or rFC for that assay.

The product, at an appropriate dilution less than the MVD, is “spiked” with endotoxin that should fall near the middle of the standard curve (currently **50**, 5, 0.5, 0.05 and 0.005 EU/ml (KLAL) and 5, 0.5, 0.05 & 0.005 EU/ml (rFC) for most assays). Acceptance criteria include the requirement that the recovery of the spike be within 50% and 200% of the expected value.

Once it is established that there is no interference by the product at this dilution, the results are independently checked, then the routine test dilution is entered into Bio-BEE-Form-40 – Product

Specific Details for Endotoxin Testing. This is the dilution at which the product will be tested unless validated otherwise.

An ideal product concentration is informative with respect to the company specification or protocol results, and well within the MVD. The product can be tested to the MVD if more concentrated testing cannot be performed due to inhibition or enhancement of the reaction or due to expected endotoxin contamination problems.

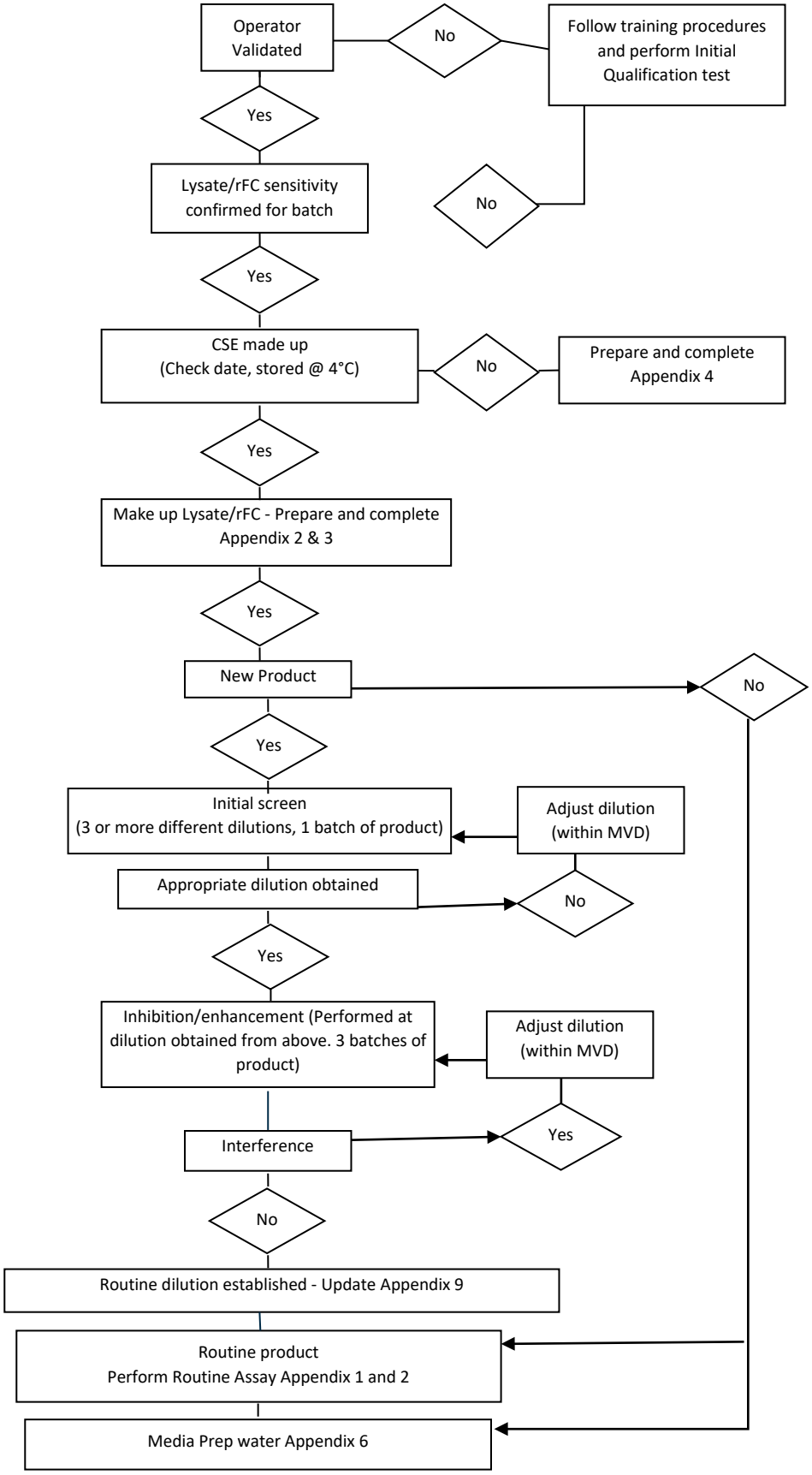
The Biotherapeutics Section also performs endotoxin testing on water samples submitted by the Media Preparation Unit. The test is validated on both the LAL and rFC methods and is outlined in Bio-BEE-Form-35 - Media Preparation Water Endotoxin Testing.

Method

The different types of endotoxin assays and the associated methods performed within this laboratory.

- Bio-BEE-Method 1 – Initial Qualification and Operator Qualification
- Bio-BEE-Method 2 – Routine Endotoxin Assay
- Bio-BEE-Method 3 – Bexsero Endotoxin Assay
- Bio-BEE-Method 4 – Endotoxin Software and Equipment Use
- Bio-BEE-Method 5 – Preparation of Control Standard Endotoxin (CSE)
- Bio-BEE-Method 6 – Endotoxin Reagents Preparation
- Bio-BEE-Method-7 – Performing an Endotoxin Assay

A brief outline of the endotoxin testing process is provided below:


Operator Validated
No
Follow training procedures and perform Initial Qualification test
Yes
Lysate/rFC sensitivity confirmed for batch
No
Yes
CSE made up (Check date, stored @ 4°C)
No
Prepare and complete Appendix 4
Yes
Make up Lysate/rFC - Prepare and complete Appendix 2 & 3
Yes
New Product
No
Yes
Initial screen (3 or more different dilutions, 1 batch of product)
Adjust dilution (within MVD)
Appropriate dilution obtained
No
Yes
Inhibition/enhancement (Performed at dilution obtained from above. 3 batches of product)
Adjust dilution (within MVD)
Interference
Yes
No
Routine dilution established - Update Appendix 9
Routine product
Perform Routine Assay Appendix 1 and 2
Media Prep water Appendix 6

Equipment and Materials

All equipment used in the KLAL or *rFC* assays must be free of detectable endotoxin and of interfering effects for the assay. Careful technique is also required to avoid endotoxin contamination.

Glassware that is not purchased as certified pyrogen free can be depyrogenated in the hot air oven at 250°C for not less than 1 hour (performed by the Media Preparation & Sterilisation Unit within the Microbiology Section).

The following equipment and materials are required to perform the endotoxin assays:

- Lonza Endotoxin Detection System – Biotek or SpectraMax Plate Reader and WinKQCL software
- Pipettors – 10 µl, 200 µl & 1000 µl adjustable pipettes and 100 µl multichannel pipette - must be within calibration period and be checked with the correct pyrogen-free tips
- Pipette tips – currently Eppendorf Biopur purchased from and certified pyrogen-free by Lonza
- Tubes - purchased from and certified pyrogen-free by Lonza
- Reagent reservoir (purchased from and certified pyrogen-free by Lonza)
- Pyrogen-free 13 x 100 mm borosilicate glass tubes (purchased or depyrogenated in-house)
- KLAL Plates (purchased from and certified pyrogen-free by Lonza – currently Costar 3596)
- *rFC* Plates (Corning® 96 Well Black Polystyrene Microplate purchased from Merck Cat# CLS3603)
- Lonza Limulus Amoebocyte Lysate Kinetic-QCL Kit – containing LAL Reagent, Control Standard Endotoxin and LAL Reagent Water or
- Pyrosperse™ Dispersing Agent (Used on specifically validated assays with the KQCL Kit Only)
- Lonza Pyrogene™ Recombinant Factor C Kit – containing rFC Enzyme Solution, Fluorogenic Substrate, rFC Assay Buffer, Control Standard Endotoxin and LAL Reagent Water
- Depyrogenated black lid screw-top bottles or re-used tubes (depyrogenation is in-house) or other suitable, pyrogen-free, storage vessel
- Forceps, scissors, Parafilm, racks, timer, marker pens, waste bag and vortex mixer

The commercially available reagents are supplied within the kit. We are currently using LONZA reagents (KLAL KQCL kit Cat# 50-650U, rFC Pyrogene™ kit Cat# 50-658U) but could substitute for another of the FDA licensed manufacturers. The reagents are used according to the manufacturer's instructions. The computer software will only accept matched reagents, so reagents from one kit cannot be mixed with reagents from another.

Precautions

- Ensure that the correct (**endotoxin free**) tips, tubes, plates etc are used.
- Careful technique is required to avoid endotoxin contamination.
- The times and temperatures used in this assay must be adhered to.
- Fill out the appropriate forms from the Quality Management System.
- The method should be carried out as per this SOP, which is based on the manufacturer's instructions in the relevant Lonza kit insert and on pharmacopoeial methods.
- Please read ALL of this SOP and the relevant methods before beginning the assay.

The outcome of the assay results depends heavily upon the timing and technique of adding the lysate or rFC mixture to the reaction plate. This is especially evident when performing an Initial Qualification assay due to the quadruplicate nature of the assay. This means that the remainder of the assay method is largely about the preparation leading up to this important step.

Setting up the Software

When performing an endotoxin assay, follow the procedures for the use of the software as described in Bio-BEE-Method 4.

Preparation of Control Standard Endotoxin (CSE)

Prepare the CSE as described in Bio-BEE-Method–5, with reference to the details stated on the Certificate of Analysis.

The next steps to be performed are dependent upon the type of assay, and are divided into separate methods, Initial Qualification, Routine Assay and Bexsero Assay. Refer to the most appropriate method based on the type of assay you are performing.

Qualification Assay

If performing a qualification assay, quadruplicate aliquots of the standards are added to the plate, follow the procedures as described in Bio-BEE-Method-1

Routine Assay

If performing a routine assay, duplicate aliquots of the standards are added to the plate, follow the procedures as described in Bio-BEE-Method-2.

Bexsero Assay (Kinetic LAL)

If performing a Bexsero assay, duplicate aliquots of a 3-point standard curve are added to the plate, follow the procedures as described in Bio-BEE-Method-3.

Preparation of Sample Dilutions

If you are performing an assay to test samples (using a Routine Assay or a Bexsero Assay) you will also need to set up the appropriate tubes for predilution and testing of samples before proceeding.

Sample dilutions are prepared in pyrogen free tubes or bottles. Dilution of samples to the routine test dilutions are recorded on Bio-BEE-Form-42.

Sample Type

For testing most samples and Media Preparation Unit water samples, refer to the routine sample method (Bio-BEE-Method-2). Bexsero samples require special treatment. If testing Bexsero samples, please refer to the Bexsero method (Bio-BEE-Method-3).

Starting and Completing the Assay

Depending on the endotoxin assay being performed refer to Bio-BEE-Method-1, Bio-BEE-Method-2 or Bio-BEE-Method-3. Prepare the software for the reaction as set out in Bio-BEE-Method-4. Prepare the required reagents and materials as set out in Bio-BEE-Method-6. How to perform an endotoxin assay is described in Bio-BEE-Method-7.

Assay Acceptance Criteria

The standard curve must meet the following parameters for the assay to be considered valid.

- Correlation coefficient (r) absolute value ≥ 0.980
- Slope between -0.400 and -0.100 (KLAL) or *0.760 and 1.110 (rFC)*
- Y intercept between 2.500 and 3.500 (KLAL) or *2.500 and 5.000 (rFC)*
- Mean reaction times of blank ≥ mean reaction times of lowest standard (KLAL)
- *Mean RFU of blank ≤ mean RFU of lowest standard (rFC)*
- Coefficient of variation (CV) values for all standards are < 10% (KLAL) or *< 25% (rFC)*

The product passes the test if the endotoxin content is below the specified limit for the product. The WinKQCL software will take the dilution factor of the sample into account and calculate the amount of endotoxin in the product. This can then be compared to the endotoxin limit to determine whether the product meets the requirements of the test.

Conclusions & Recording Results

The results are stored as part of the assay in the WinKQCL software. A complete record of each test is maintained by completing the appropriate worksheet and results sheets for the particular assay. To complete these sheets, transcribe the results into them i.e., % CV's, acceptance criteria parameters, EU/ml, and % PPC Recovery. These sheets form a permanent record of each test and are archived for future reference in the endotoxin testing folders. All records are stored electronically, so any hardcopy records should be scanned into TRIM (e.g., E22-500821 - Biotherapeutics Section - Bacterial Endotoxin - Assays and results – 2022) **or** (E22-500844 - Biotherapeutics Section - Bacterial Endotoxin - Recombinant Factor C (rFC) - Assays and results – 2022).

Associated Documents

- Bio-BEE-Method-1 – Initial and Operator Qualification Endotoxin Assay
- Bio-BEE-Method-2 – Endotoxin Routine Assay
- Bio-BEE-Method-3 – Bexsero Endotoxin Assay
- Bio-BEE-Method-4 – Software and Equipment Use
- Bio-BEE-Method-5 – CSE Preparation
- Bio-BEE-Method-6 – Reagent Preparation
- Bio-BEE-Method-7 – Performing an Endotoxin Assay
- Bio-BEE-Form-34 – Bexsero Assay Worksheet
- Bio-BEE-Form-35 – Media Preparation Water Endotoxin Testing
- Bio-BEE-Form-36 – Initial and Operator Qualification Worksheet
- Bio-BEE-Form-37 – CSE Preparation
- Bio-BEE-Form-38 – Bacterial Endotoxin Kinetic Lysate Preparation
- Bio-BEE-Form-39 – Endotoxin Routine Assay Worksheet
- Bio-BEE-Form-40 – Product Specific Details for Endotoxin Testing
- Bio-BEE-Form-42 – Endotoxin Routine Assay Sample Results Sheet

Australian Government
Department of Health and Aged Care
Therapeutic Goods Administration

Laboratories Branch

Owner: s22 [redacted]	**Number:** Bio-BPC-Form-18
Author: s22 [redacted]	**Version:** 1
Active:	**Review:** 21/04/2023
Title: Fragment Analyzer – Worksheet - General	

Worksheet for Fragment Analyzer

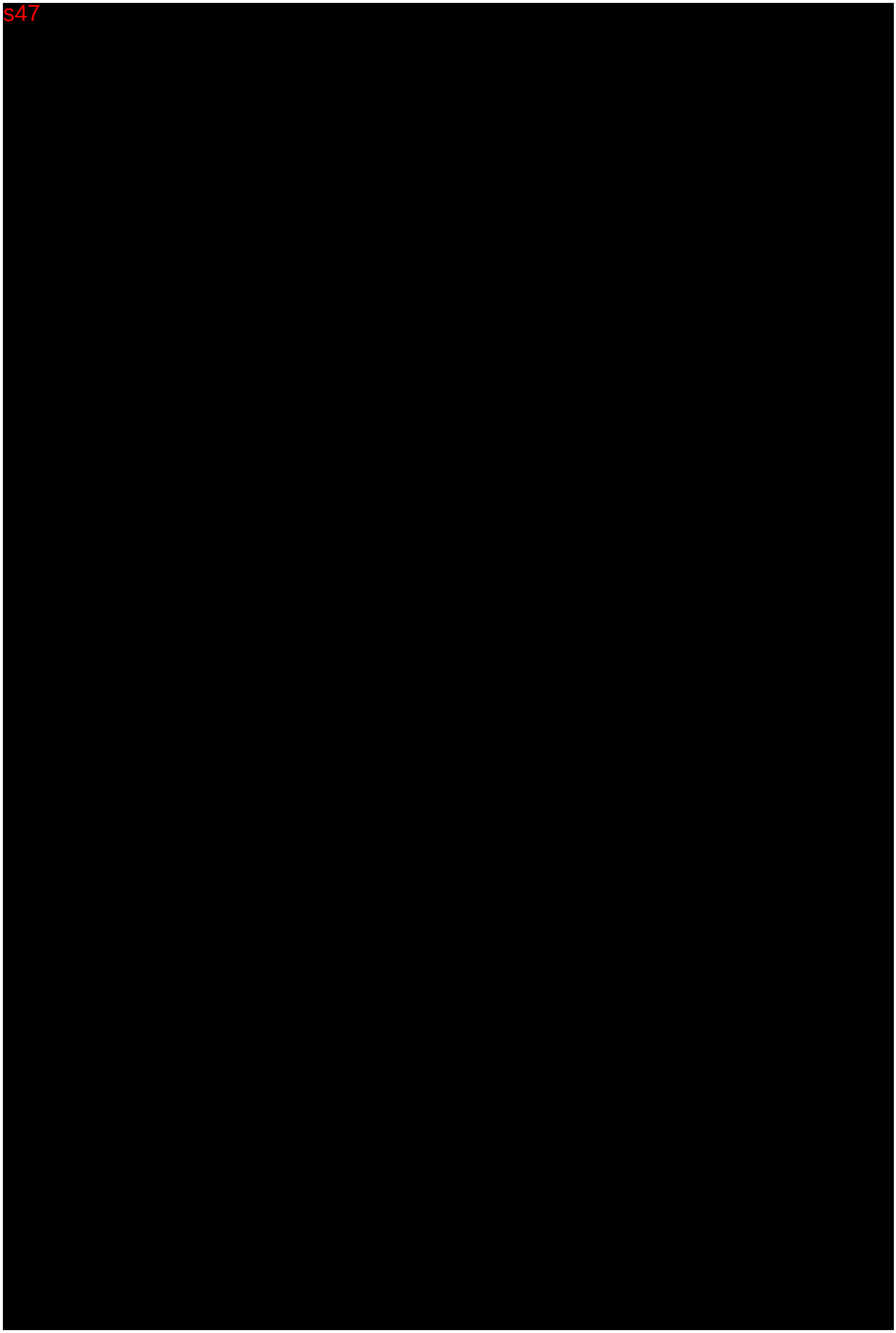
Test Details			
SOP QPulse #	Bio-BPC-Method-31	**Analyst**	s22 [redacted]
TRIM link to data files	el://E21-319137?db=A7&open	**Test Date**	23/11/2022
Modifications to SOP	1. Using a thermomixer instead of thermocycler: D21-3185919		

Pipettes & Equipment	
Name	**LIMS#**
P20	32677
P1000	5643
P200	5649
P10	32835
Thermomixer	Enter text.
Enter text.	Enter text.
Enter text.	Enter text.
Enter text.	Enter text.

Reagents & Consumables			
Details	**Catalog #**	**Lot/Batch Number**	**Expiry date**
48-Capillary Array, short 33 cm	Enter text.	Enter text.	Enter a date.
Inlet Buffer	DNF-355-0300	6654278	8/12/2022
Rinse Buffer	DNF-497-0125	6678610	18/04/2023
Capillary Storage Buffer	Enter text.	6581286	28/12/2022
Capillary conditioning solution	DNF-475-0100	6656325	14/12/2022
RNA Separation Gel	DNF-265-0500	6655702	9/01/2023
Intercalating dye	DNF-600-U030	6651732	23/11/2022
Blank	DNF-300-0008	6681489	2/05/2023
RNA Ladder	DNF-382-U020	6650691	1/07/2023
Diluent Marker	DNF-369-0004	6602442	7/04/2023
DEPC water	Enter text.	20220315/01	15/03/2023
20% T-X100 / 30% EtOH solution	Enter text.	MC23Aug22-01	23/11/2022
Enter text.	Enter text.	Enter text.	Enter a date.
Enter text.	Enter text.	Enter text.	Enter a date.
Enter text.	Enter text.	Enter text.	Enter a date.

Reagent Preparation			
REAGENT	STORAGE	Date Prepared	Expiry
s47 [redacted]			

s47



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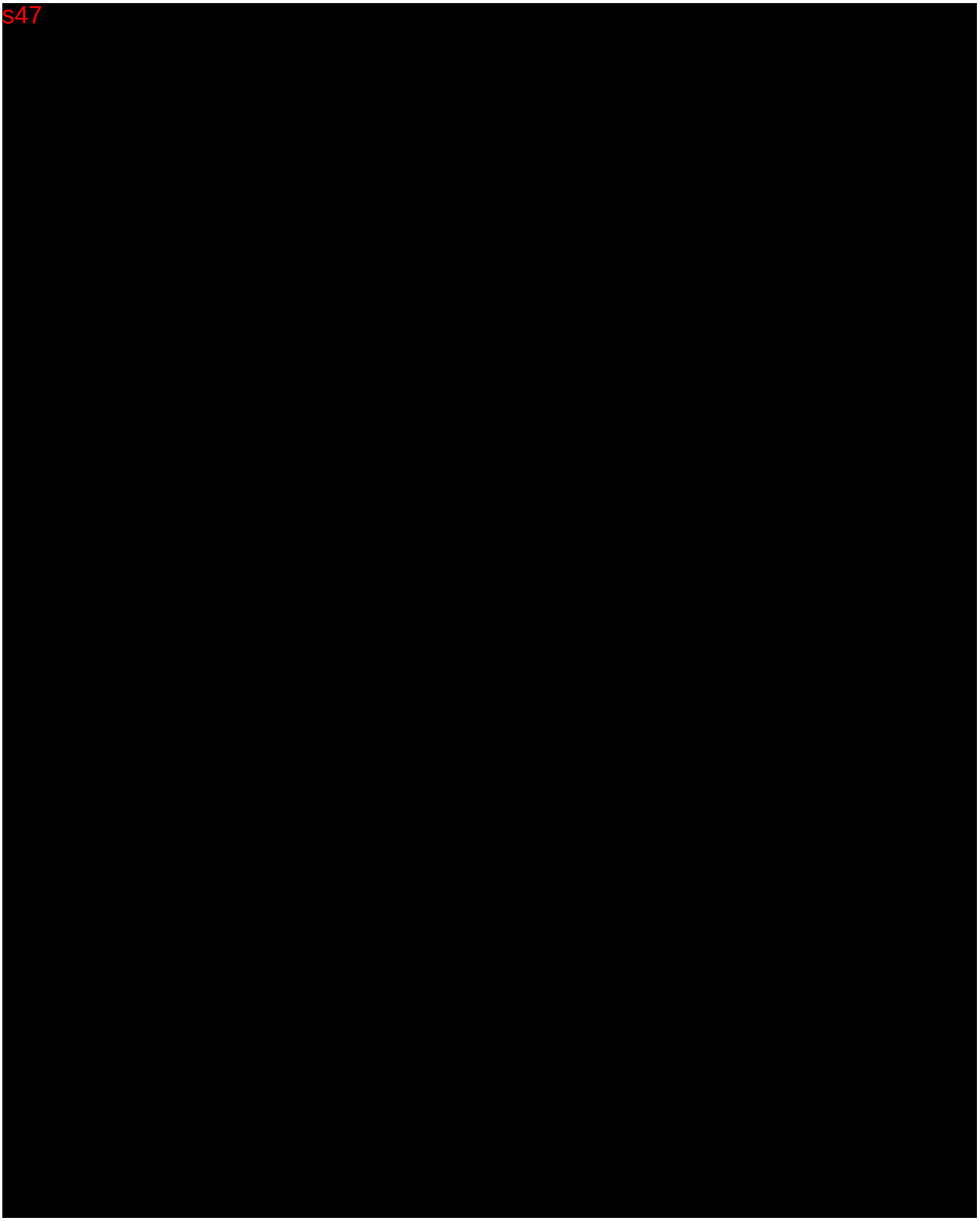

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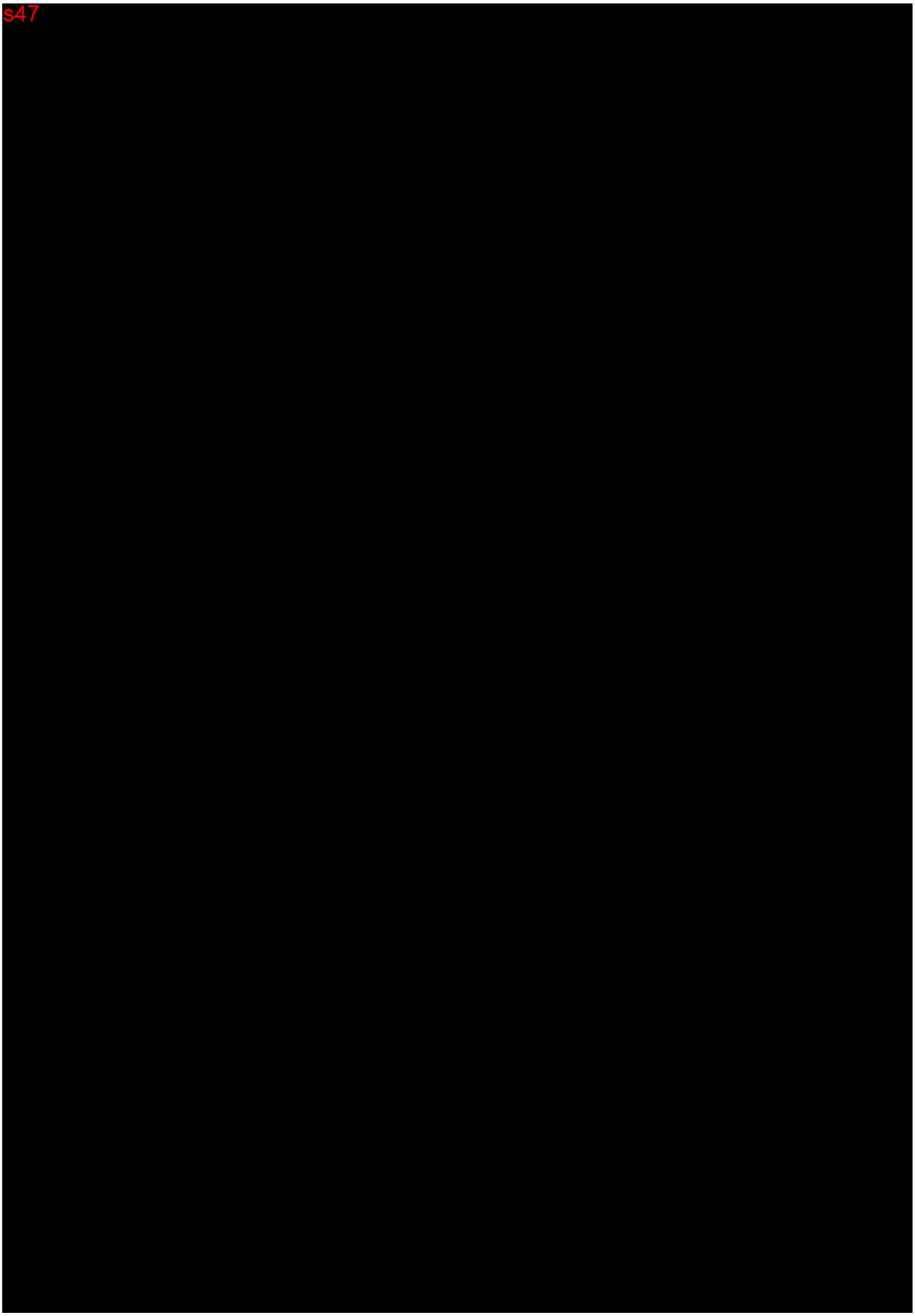


s47 [redacted]

Sample Results	
PASS	
Analysist	[redacted]
Checked by	[redacted]
Sample Dilutions / Calculation / Notes	

s47 [redacted]

Sample 3	
s47 [redacted]	
BATCH #	GD6794
s47 [redacted]	
Sample Results	
PASS	
Analysist	[redacted]
Checked by	[redacted]
Sample Dilutions / Calculation / Notes	
s47 [redacted]	

Sample 4	
s47 [redacted]	
BATCH #	GE1643
s47 [redacted]	
Sample Results	
PASS	
Analysist	[redacted]
Checked by	[redacted]
Sample Dilutions / Calculation / Notes	
s47 [redacted]	

Sample 5	
s47 [redacted]	
BATCH #	GE8382

s47 [redacted]

Sample Results
PASS

Analysist	[redacted]
Checked by	[redacted]

Sample Dilutions / Calculation / Notes
s47 [redacted]

Sample 6	
Plate location (wells)	Choose an item.
LIMS #	**Click or tap here to enter text.**
BATCH #	Click or tap here to enter text.
EXPIRY	Enter date.

Sample Acceptance Criteria			
Parameter	**Limits**	**Results**	**Comments**
Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Choose an item.

Test Results					
Parameters	**Limits**	**Results**			**Comments**
		Average	**SD**	**%RSD**	
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.

Sample Results	
Choose an item.	
Analysist	Enter text.
Checked by	Enter text.

Sample Dilutions / Calculation / Notes
Enter text.

Notes
Enter text.

Australian Government
Department of Health and Aged Care
Therapeutic Goods Administration

Laboratories Branch

Owner: s22	**Number:** Chem-Form-62
Author: s22	**Version:** 1
Active: 15/06/2021	**Review:** 15/12/2022
Title: Assay for Lipids in Vaccines by HPLC CAD (Bio-BPC-Method-24 Pfizer)	

TGA SAMPLE No. 2211003542 **SUBSTANCES ASSAYED: ALC-0159, Cholesterol,**

Lot number: GE1643 **ALC-0315, DSPC**

METHOD REFERENCE: TM100010322 (Pfizer)/~~BIO BPC Method 24~~ Chem-Method-54 s22 1/12/22

INSTRUMENT OR SYSTEM No. 20 with CAD detector… Method Modifications approved by: s22 1/12/22

SYSTEM SUITABILITY REQUIREMENTS MET (Y/~~N~~) ASSAY REQUIREMENTS MET (Y/~~N~~)

RESULTS Strikethrough to specification made by s22 1/12/22 (Specification for bivalent formulation) s22 1/12/22

TGA Sample number	Preparation	Lipid content (mg/mL)			
		ALC-0159 ~~(0.55 to 1.20)~~ (0.11 – 0.24)	Cholesterol ~~(1.80 to 3.90)~~ (0.36 – 0.78)	ALC-0315 ~~(4.50 to 9.25)~~ (0.90 - 1.85)	DSPC ~~(0.90 to 2.05)~~ (0.18 – 0.41)
2211003542 Corrections made to include purity s22 01/12/2022	1	s47 [redacted]			
	2				
	3				
Average	-				
% RSD (Requirement NMT 10%)	-				

ATTACHMENTS: *Analysts Notes worksheets* ☑ *Result summary report* ☑ *System suitability report* ☑ *Example chromatograms* ☑ *Run and method summary* ☑ Calibration plots ☑ MP preparation details ☑ or refer to sample ~~D22-6185836~~ D22-6187812 s22 01/12/22, D22-6180929 and D22-6186482 Other ☐……

REQUIREMENTS (Manuf.) ~~ALC-0159 0.55 to 1.20 mg/mL; Cholesterol 1.80 to 3.90 mg/mL; ALC-0315 4.50 to 9.25 mg/mL;DSPC 0.90 to 2.05 mg/mL~~ ALC0159 0.11-0.24 mg/mL; cholesterol 0.36-0.78 mg/mL; ALC-0315 0.90-1.85 mg/mL; DSPC 0.18-0.41 mg/mL s22 1/12/22 Correct Requirement for bivalent formulation

Identification RT requirements met ? (RT ± 5% compared to SST**) (Y/~~N~~)**

IDENTIFIED AS ALC-0159, Cholesterol, ALC-0315 and DSPC

RESULT **PASS** ☑ **FAIL** ☐

Signature of Analyst Signed electronically by s22 Date 01/12/2022

Checked by Official Analyst…Signed electronically by s22 …….Date……2/12/22…

WORKING STANDARD CURVE PREPARATION DETAILS

Date Stock solution prepared: 28 Nov 2022 **Prepared by:** s22 [redacted] **Within expiry** Y/~~N~~

Attached ☐ **or Refer to (TRIM Record number/Sample number)** D22-6180792

	ALC-0159	Cholesterol	ALC-0315	DSPC5
~~Concentration in stock solution (mg/mL)~~ s22 01/12/2022	s47 [redacted]			
Corrected with purity Concentration in stock solution (mg/mL) s22 01/12/2022				

Prepared by: s22 [redacted] Date prepared 28 Nov 2022 POVA LIMS: 33271

Level	Mixed Lipid Soln (uL)	MeOH (uL)	DF	Final Target conc (mg/mL)	~~Actual conc (mg/mL)~~ s22 01/12/2022	Corrected Actual conc with purity (mg/mL) s22 01/12/22
1	50	950	20	ALC-0159	s47 [redacted]	
				Cholesterol		
				ALC-0315		
				DSPC		
2	100	900	10	ALC-0159		
				Cholesterol		
				ALC-0315		
				DSPC		
3	100	300	4	ALC-0159		
				Cholesterol		
				ALC-0315		
				DSPC		
4	200	200	2	ALC-0159		
				Cholesterol		
				ALC-0315		
				DSPC		
5	225	75	1.33	ALC-0159		
				Cholesterol		
				ALC-0315		
				DSPC		

ASSAY CONTROL PREPARATION DETAILS

Date control solution prepared: 28 Nov 2022 **Within expiry** Y/~~N~~

Attached ☑ **or Refer to (TRIM/ Record number/Sample number)** D22-6180792

DP REFERENCE MATERIAL PREPARED ~~Y~~/N s22 1/1/2/22

LIMS number	Lot number/Expiry		Sample solution (uL)	MeOH (uL)

SAMPLE PREPARATION DETAILS

Date prepared: 28 Nov 2022 **Prepared by:** s22 [redacted] **POVA LIMS:** **33271**

Preparation	Sample solution (uL)	MeOH (uL)	DF DF 10 added by s22 [redacted] 1/12/22
1	100	900	~~20~~ 10
2	100	900	~~20~~ 10
3	100	900	~~20~~ 10

Methanol LCMS grade; Supplier: Fisher Lot No 205074

METHOD MODIFICATIONS

s47 [redacted]

Australian Government
Department of Health and Aged Care
Therapeutic Goods Administration

Laboratories Branch

Type: Biotherapeutics\BEE\Forms	**Number:** Bio-BEE-Form-42 / **Version:** 3
Owner: s22 [redacted]	**Approver:** s22 [redacted]
Active: 10/05/2022	**Review:** 10/11/2023
Title: Endotoxin Routine Assay Sample Results	

Endotoxin Routine Assay Sample Results

Assay ID: 22Nov2022 Operator: s22 [redacted]

LAL Reagent Water (LRW) Lot Number: 0000966190 LRW Expiry: 20Jul2023

Other Reagent: Pyrosperse Batch# 0000981194 Expiry: 17Mar2023 Use By: 19Dec2022

This form is used for recording the sample results. Use Bio-BEE-SOP 28 and appropriate method for the detailed procedures.

1 - Product Details (refer to Bio-BEE-Method-8, where required)

Sample Number	Product Name	Batch Number	Expiry	LIMS Number	Endotoxin Limit EU/mL	Test Dilution
1	Pfizer (Bivalent Original/Omicron)	s22 [redacted]				
2	Pfizer (Bivalent Original/Omicron)					
3	Pfizer (Bivalent Original/Omicron)	GE8382	31May2023	2211003544	s47 [redacted]	1000 (with Pyrosperse™ at 1/200 (v/v)
4	Pfizer (Bivalent Original/Omicron)	GE1643	30Apr2023	2211003542		1000 (with Pyrosperse™ at 1/200 (v/v)
5	Pfizer (Bivalent Original/Omicron)	GD6794	30Apr2023	2211003540		1000 (with Pyrosperse™ at 1/200 (v/v)
6	n/a	-	-	-	-	-
7	n/a	-	-	-	-	-
8	n/a	-	-	-	-	-
9	n/a	-	-	-	-	-
10	n/a	-	-	-	-	-
11	n/a	-	-	-	-	-

2 - Product Dilutions

Sample Number	Dilution Steps	Dilution	Volume of Dilution	Volume of LRW (µL)	Volume of Other (µL)
1	s22				
2					
3	3.1	1/50	20uL of vaccine	975	5
	3.2	1/20	50uL of 1/50 dilution	945	5
	3.3	n/a	-	-	-
4	4.1	1/50	20uL of vaccine	975	5
	4.2	1/20	50uL of 1/50 dilution	945	5
	4.3	n/a	-	-	-
5	5.1	1/50	20uL of vaccine	975	5
	5.2	1/20	50uL of 1/50 dilution	945	5
	5.3	n/a	-	-	-
6	6.1	n/a	-	-	-
	6.2	n/a	-	-	-
	6.3	n/a	-	-	-
7	7.1	n/a	-	-	-
	7.2	n/a	-	-	-
	7.3	n/a	-	-	-
8	8.1	n/a	-	-	-
	8.2	n/a	-	-	-
	8.3	n/a	-	-	-
9	9.1	n/a	-	-	-
	9.2	n/a	-	-	-
	9.3	n/a	-	-	-
10	10.1	n/an/a	-	-	-
	10.2	n/a	-	-	-
	10.3	n/a	-	-	-
11	11.1	n/a	-	-	-
	11.2	n/a	-	-	-
	11.3	n/a	-	-	-

Recording of Results

The results are calculated by the WinKQCL software. Transcribe results from the WinKQCL report to assay sheet.

Sample Number	Test CV (%)	Result (EU/ml)	PPC CV (%)	% PPC Recovery
1	s22			
2				
3	N/A (Undefined)	s47		
4	N/A (Undefined)			
5	N/A (Undefined)			
6	n/a	-	-	-
7	n/a	-	-	-
8	n/a	-	-	-
9	n/a	-	-	-
10	n/a	-	-	-
11	n/a	-	-	-

For the sample test to be valid, the PPC recovery must be 50-200% of the expected value and the coefficient of variation (CV) of the sample and PPC duplicates should be < 10% (LAL) or < 25% (*rFC*). Many samples do not reach an endpoint and are not assigned a %CV. In these instances, contact the person in charge of the assay.

Validity Criteria		
Were the standard curve validity criteria met?		Yes
Sample Number	Were the sample validity criteria met?	Was the result within the endotoxin limit?
1	s22	
2		
3	Yes	Yes
4	Yes	Yes
5	Yes	Yes
6	n/a	n/a
7	n/a	n/a
8	n/a	n/a
9	n/a	n/a
10	n/a	n/a
11	n/a	n/a

Notes:

Checked s22 **24Nov2022**

Australian Government
Department of Health and Aged Care
Therapeutic Goods Administration

Laboratories Branch

Owner: s22 [redacted]	**Number:** Bio-BPC-Form-14
Author: s22 [redacted]	**Version:** 1
Active: 13/07/2021	**Review:** <QPulse_DocReviewDate>
Title: Zetasizer Worksheet	

Worksheet for Zetasizer – DLS – Particle Size and polydispersity

Test Details			
SOP QPulse #	Bio-BPC-Method-27	**Analysist**	s22 [redacted]
TRIM link to data files	E21-331895	**Test Date**	22/11/2022

Pipettes & Equipment	
Name	**LIMS#**
P10	33249
P200	32190
P1000	5705
Enter text.	Enter text.
Enter text.	Enter text.
Enter text.	Enter text.

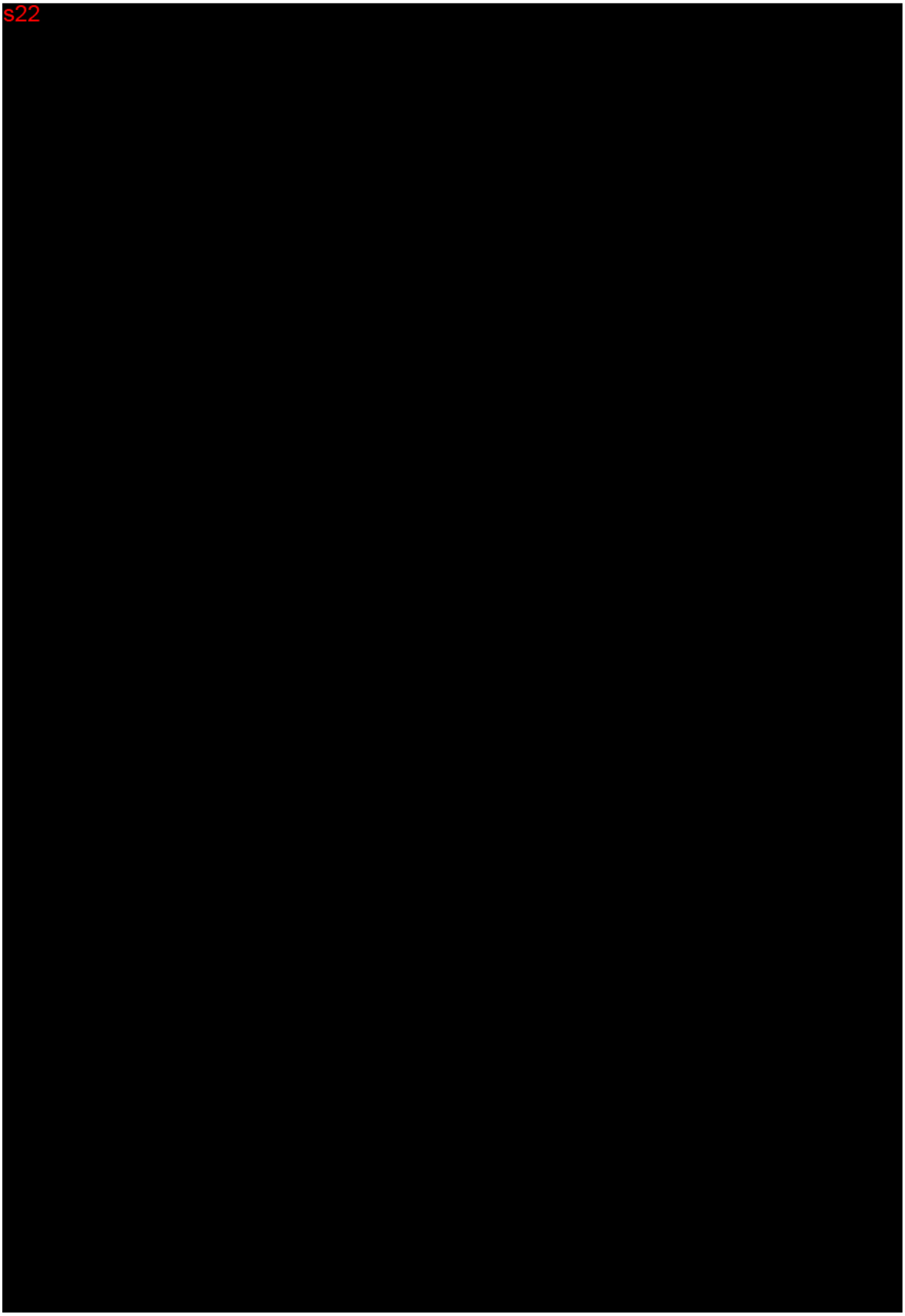
Reagents & Consumables			
Details	**Catalog #**	**Lot/Batch Number**	**Expiry date**
Nanosphere size standard 150 nm	3150A	236572	28/03/2024
Reference Material	PF controls	PF-07302048_EL8983	3/12/2022
PBS	In house	20210729/01	3/03/2023
Cuvettes	DTS0012	1005/1007004	N/A
Enter text.	Enter text.	Enter text.	Enter a date.
Enter text.	Enter text.	Enter text.	Enter a date.

Zetasizer Performance Test				
Size Standard	**Parameter**	**Limits**	**Results**	**Comments**
150 nm	Average of Mean hydrodynamic diameter (nm)	140-160 nm	155.9	PASS
150 nm	%RSD of mean hydrodynamic diameter	≤ 10%	0.7337	PASS
System Suitability Criteria				
Product tested	**Parameter**	**Limits**	**Results**	**Comments**
150 nm standard	Mean hydrodynamic diameter (nm)	+/-10nm of CoA (142-162 nm)	155.9	PASS
150 nm standard	Mean hydrodynamic diameter (RSD)	≤ 5%	0.7337	PASS
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.

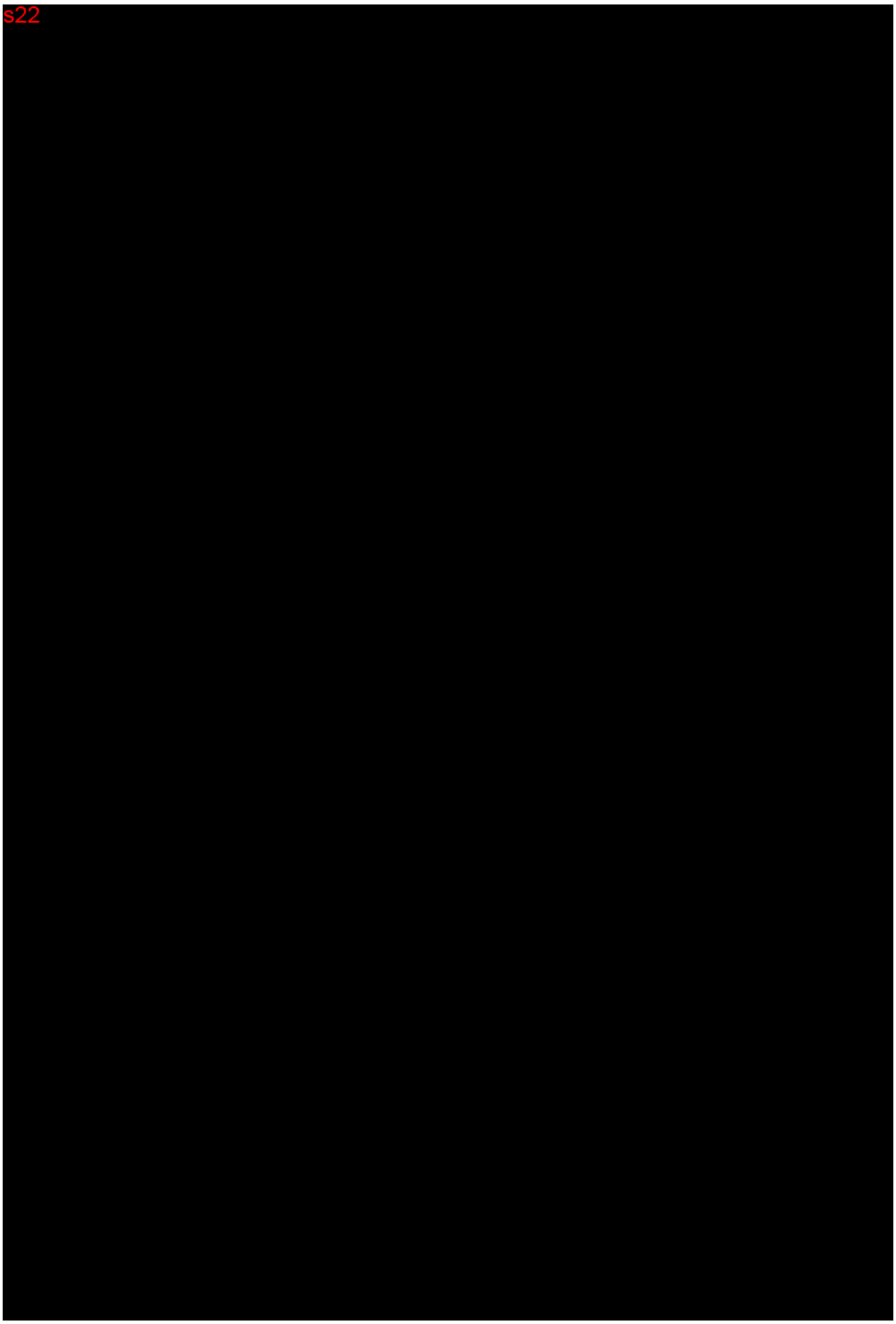
Assay Acceptance Criteria				
Product tested	**Parameter**	**Limits**	**Results**	**Comments**
150 nm standard (beginning and end of assay)	Mean hydrodynamic diameter (nm)	+/-10nm of CoA (142-162 nm)	156.1	PASS
150 nm standard (beginning and end of assay)	Mean hydrodynamic diameter (RSD)	≤ 10%	0.8234	PASS
Reference Material EL8983 211004298	Mean hydrodynamic diameter (nm)	s47 [redacted]		PASS
Reference Material EL8983 211004298	Mean hydrodynamic diameter (RSD)			PASS
Reference Material EL8983 211004298	PDI			PASS
s22 [redacted]				s22 [redacted]
Drug Product tested - 2211003544	Mean hydrodynamic diameter (RSD)			PASS
Drug Product tested - 2211003540	Mean hydrodynamic diameter (RSD)			PASS
Drug Product tested - 2211003542	Mean hydrodynamic diameter (RSD)			PASS

System suitability standard & reference material dilutions / calculation / notes
System Suitability Standard: - 5 µL of 150 nm standard added to 4995 ul of 0.1 um filtered PBS Reference Material: - 0.2 mg/mL RM --> 80 µL x 0.47 mg/mL + 108 µL PBS - 0.002 mg/mL RM --> 10 µL x 0.2 mg/mL + 990 µL PBS

s22



s22



Sample 3 Details					
LIMS #	2211003544				
BATCH #	GE8382				
EXPIRY	31/05/2023				
Test Results					
Parameters	**Limits**	**Parameters**			**Limits**
		Average	**SD**	**%RSD**	
Mean hydrodynamic diameter (nm)	s47 [redacted]				PASS
Polydispersity Index					PASS
Result quality	Good	Good			PASS
Sample Dilutions / Calculation / Notes					
0.2 mg/mL DP --> 80 µL x 0.5 mg/mL + 120 µL PBS 0.002 mg/mL DP --> 10 µL x 0.2 mg/mL + 990 µL PBS					
Sample Results					
PASS					
Analysist	[redacted]				
Checked by	s22 [redacted]				

Sample 4 Details	
LIMS #	2211003540
BATCH #	GD6794
EXPIRY	30/04/2023

Test Results

Parameters	Limits	Results			Comments
		Average	SD	%RSD	
Mean hydrodynamic diameter (nm)	s47 [redacted]				PASS
Polydispersity Index					PASS
Result quality	Good	Good			PASS

Sample Dilutions / Calculation / Notes

0.2 mg/mL DP --> 80 µL x 0.5 mg/mL + 120 µL PBS
0.002 mg/mL DP --> 10 µL x 0.2 mg/mL + 990 µL PBS

Sample Results

Choose an item.

Analysist	s22 [redacted]
Checked by	s22 [redacted]

Sample 5 Details					
LIMS #	2211003542				
BATCH #	GE1643				
EXPIRY	30/04/2023				
Test Results					
Parameters	**Limits**	**Results**			**Comments**
		Average	**SD**	**%RSD**	
Mean hydrodynamic diameter (nm)	s47 [redacted]				PASS
Polydispersity Index					PASS
Result quality	Good	Good			PASS

Sample Dilutions / Calculation / Notes
0.2 mg/mL DP --> 80 µL x 0.5 mg/mL + 120 µL PBS 0.002 mg/mL DP --> 10 µL x 0.2 mg/mL + 990 µL PBS

Sample Results	
Choose an item.	
Analysist	s22 [redacted]
Checked by	s22 [redacted]

Sample 6 Details	
LIMS #	Click or tap here to enter text.
BATCH #	Click or tap here to enter text.
EXPIRY	Enter date.

Test Results

Parameters	Limits	Results			Comments
		Average	SD	%RSD	
Mean hydrodynamic diameter (nm)	s47 [redacted]	Enter text.	Enter text.	Enter text.	Choose an item.
Polydispersity Index		Enter text.	Enter text.	Enter text.	Choose an item.
Result quality	Enter text.	Enter text.			Choose an item.

Sample Dilutions / Calculation / Notes

Enter text.

Sample Results

Choose an item.

Analysist	Enter text.
Checked by	Enter text.

Notes
Enter text

Australian Government
Department of Health
Therapeutic Goods Administration

Laboratories Branch

Operations	Biotherapeutics Laboratories Operations Manual
Procedure	PCR – Form – Quantitation of Total and Percent Encapsulated RNA in Lipid Nanoparticles by RiboGreen Fluorescence Worksheet
Written	s22 [redacted]
Authorised	s22 [redacted]
Date issued	10 August 2021
Revision #	3

Quantitation of Total and Percent Encapsulated RNA in Lipid Nanoparticles by RiboGreen Fluorescence - Worksheet

Operator	s22 [redacted]	Date of assay	24/11/2022
Checked by		TRIM link to assay data file	el://D22-6165997?db=A7&open

Results Summary

Sample Number	LIMS#
1	2211003540
2	2211003542
3	

s47 [redacted]

Reagent records

Reagent	Manufacturer and Catalogue Number	Lot Number	Expiry	Notes
Drug Substance Reference Material	PF-07305885	00711666-0110-WRM	n/a	
Quant-iT RiboGreen RNA Reagent	Thermo Fisher, R11491	2313073	n/a	
Triton X-100 10% Solution in Water	Thermo Scientific, 85111	WF322599	-	
TE Buffer	Invitrogen, AM9849	01078095	-	

Additional notes

Standard Curve Preparation

Prepare the standard curve material using aliquotted DS RM at [redacted]/mL, diluted according to the table below

Standard ID	In-Plate RNA Concentration (ng/mL)	Volume of DS RM at [redacted] mL (µL)	Volume of TE buffer (µL)
STD 1	2500	150	150
STD 2	2000	100	150
STD 3	1600	80	170
STD 4	1200	120	380
STD 5	800	100	525
STD 6	400	100	1150
STD 7	200	50	1200
STD 8	0	0	400

Drug product sample preparation - Pfizer Comirnaty

For paediatric presentation drug product, DP diluted as below;
40 uL DP + 360 uL TE buffer

Dilution factor: 62.5
Dilution factor: 250

[redacted]

Buffer Formulations

1% Triton X-100

Component	Volume Added
10% Triton X-100	3 mL
TE Buffer	27 mL

2% Triton X-100

Component	Volume Added
10% Triton X-100	6 mL
TE Buffer	24 mL

Quant-IT Ribogreen Working Solution

Component	Volume Added
Quant-IT Ribogreen Reagent	50 µL
TE Buffer	10 mL

Pipettes used for assay

32892, 32837, 32792, 33016

Document 12

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	50µL **STD 8** 50µL TE		50µL **STD 8** 50µL 2% Triton									
B	50µL **STD 7** 50µL TE		50µL **STD 7** 50µL 2% Triton		~~100µL **Sample 1** in TE buffer~~	~~100µL **Sample 2** in TE buffer~~	~~100µL **Sample 3** in TE buffer~~	TE Sample 1	TE Sample 2			
C	50µL **STD 6** 50µL TE		50µL **STD 6** 50µL 2% Triton					TE Sample 1	TE Sample 2			
D	50µL **STD 5** 50µL TE		50µL **STD 5** 50µL 2% Triton					TE Sample 1	TE Sample 2			
E	50µL **STD 4** 50µL TE		50µL **STD 4** 50µL 2% Triton		~~100µL **Sample 1** in **1% Triton** buffer~~	~~100µL **Sample 2** in **1% Triton** buffer~~	~~100µL **Sample 3** in **1% Triton** buffer~~	1% TX Sample 1	1% TX Sample 2			
F	50µL **STD 3** 50µL TE		50µL **STD 3** 50µL 2% Triton					1% TX Sample 1	1% TX Sample 2			
G	50µL **STD 2** 50µL TE		50µL **STD 2** 50µL 2% Triton					1% TX Sample 1	1% TX Sample 2			
H	50µL **STD 1** 50µL TE		50µL **STD 1** 50µL 2% Triton									

Plus 100µL of Ribogreen working solution to each assay well

After completing the assay, convert the above to .pdf and combine it with the .pdf report generated from the SoftmaxPro assay data file to form a single file containing both the worksheet and results. File the combined .pdf and the raw softmax data (.sda) in the assay specific data folder (E21-207399)

s47

Results Summary

Results Summary **LIMS# 33237** **Version 1, Feb2021** **Validated 01/02/22 - Next Due 01/02/23**

Pfizer - BNT162b2

Quantitation of Total and Percent Encapsulated RNA in PF-07302048 Lipid Nanoparticles by RiboGreen Fluorescence

Assay Validity Criteria		Observed Value	Validity
Coefficient of determination (R^2) for TE standard curve	≥ 0.98	= 0.997	VALID
Coefficient of determination (R^2) for TX standard curve	≥ 0.98	= 0.999	VALID
%RSD for all standard curve points (point 8, 0ng/mL, excluded)	≤ 20%	Pass	VALID
Monotonous increase over TE standard curve	Present	Present	VALID
Monotonous increase over TX standard curve	Present	Present	VALID

s47 [redacted]

Sample Validity Criteria:

s47 [redacted]

s47 [redacted]

Spreadsheet validation procedure:

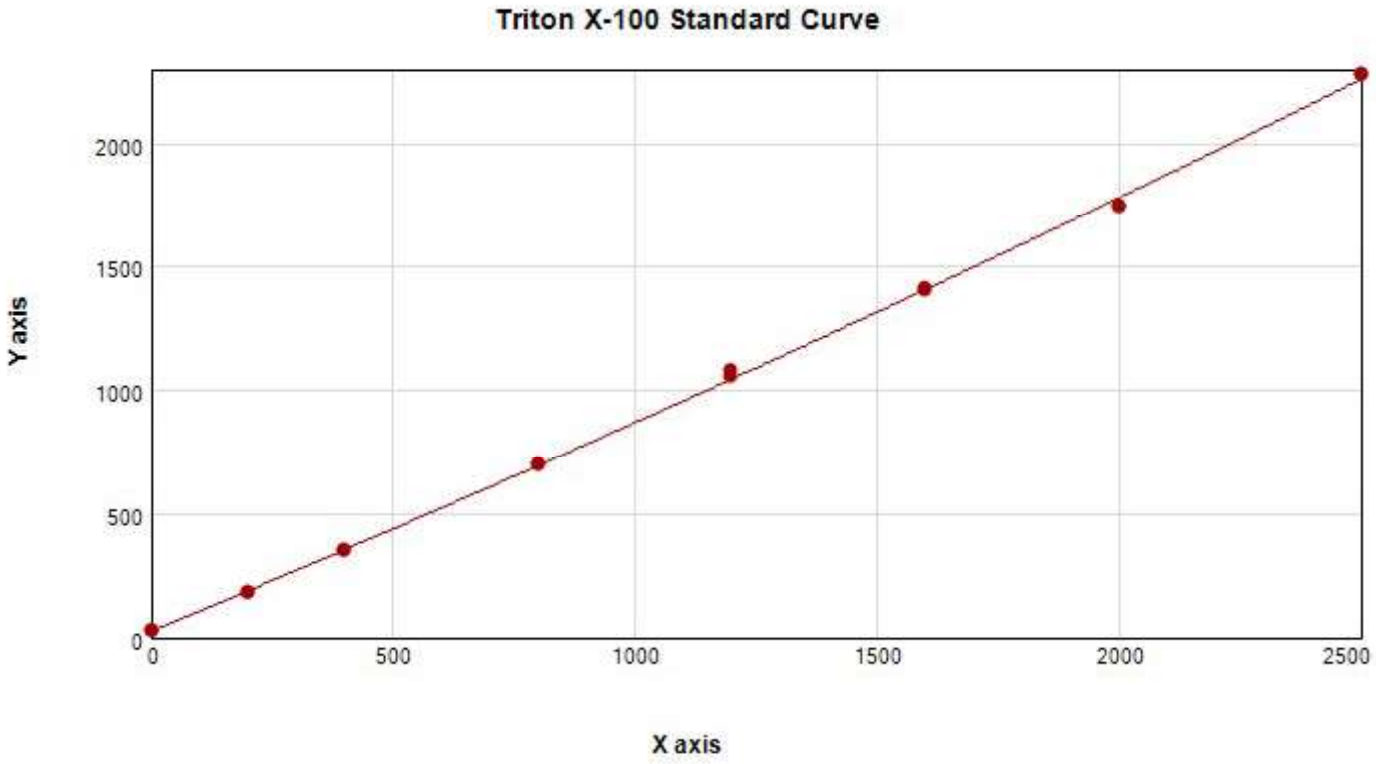
Open the active copy of Softmax Pro template "Quantitation of Total and Percent Encapsulated RNA in PF-07302048 Lipid Nanoparticles by RiboGreen Fluorescence.spr".

Open spreadsheet validation data set (TRIM link: D21-2134364) and copy well data from Plate 1. Paste well data into Plate 1 in the Softmax Pro Template undergonig validation.

Generate a .pdf printout. File the .pdf in the validation folder in TRIM (E21-212109), using thefile name "Spreadsheet validation results - LIMS#33237 - DDMMMYY - username.pdf"

If the calculated values and validity status are consistent between the generated report and those recorded in the validation dataset report (TRIM link: D21-2134332) the template is considered valid, and a "PASS" result is to be entered into qLIMS. Update the validation date and due date in the template and submit a zipped version of the template for storage in q-pulse.

In the event of inconsistent results, a "FAIL" result is to be enetered into qLIMS and the issue is to be reported to the PCR Unit Manager.

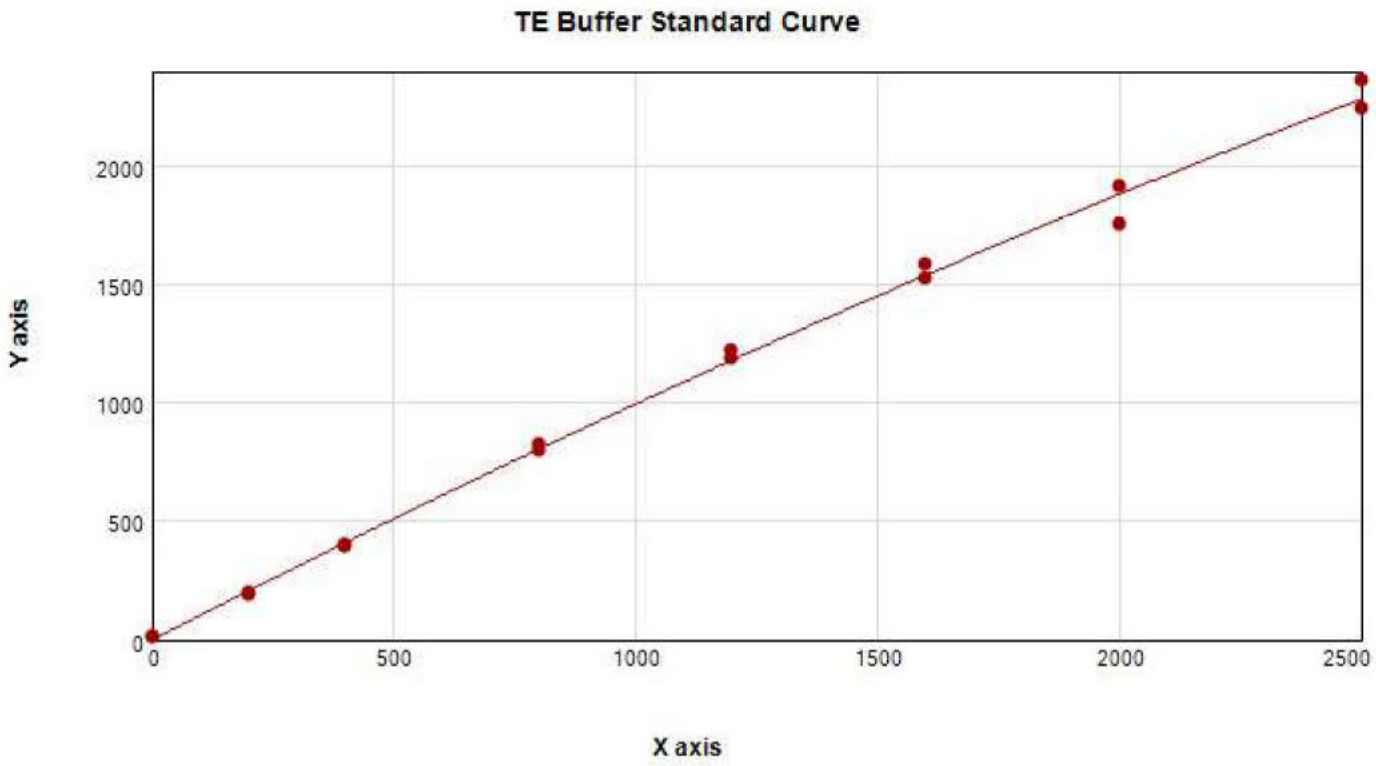


● **Plot2** (Standard Curve, Triton X-100: Values vs Concentration) Weighting: Fixed

Curve Fit Results ▲

Curve Fit : Quadratic $y = A + Bx + Cx^2$

	Parameter	Estimated Value	Std. Error	Confidence Interval
Plot2 $R^2 = 0.999$	A	26.04	9.793	[4.884, 47.20]
	B	0.812	0.020	[0.768, 0.856]
	C	3.26e-5	8.11e-6	[1.51e-5, 5.01e-5]



● **Plot1** (Standard Curve, TE Buffer: Values vs Concentration) Weighting: Fixed

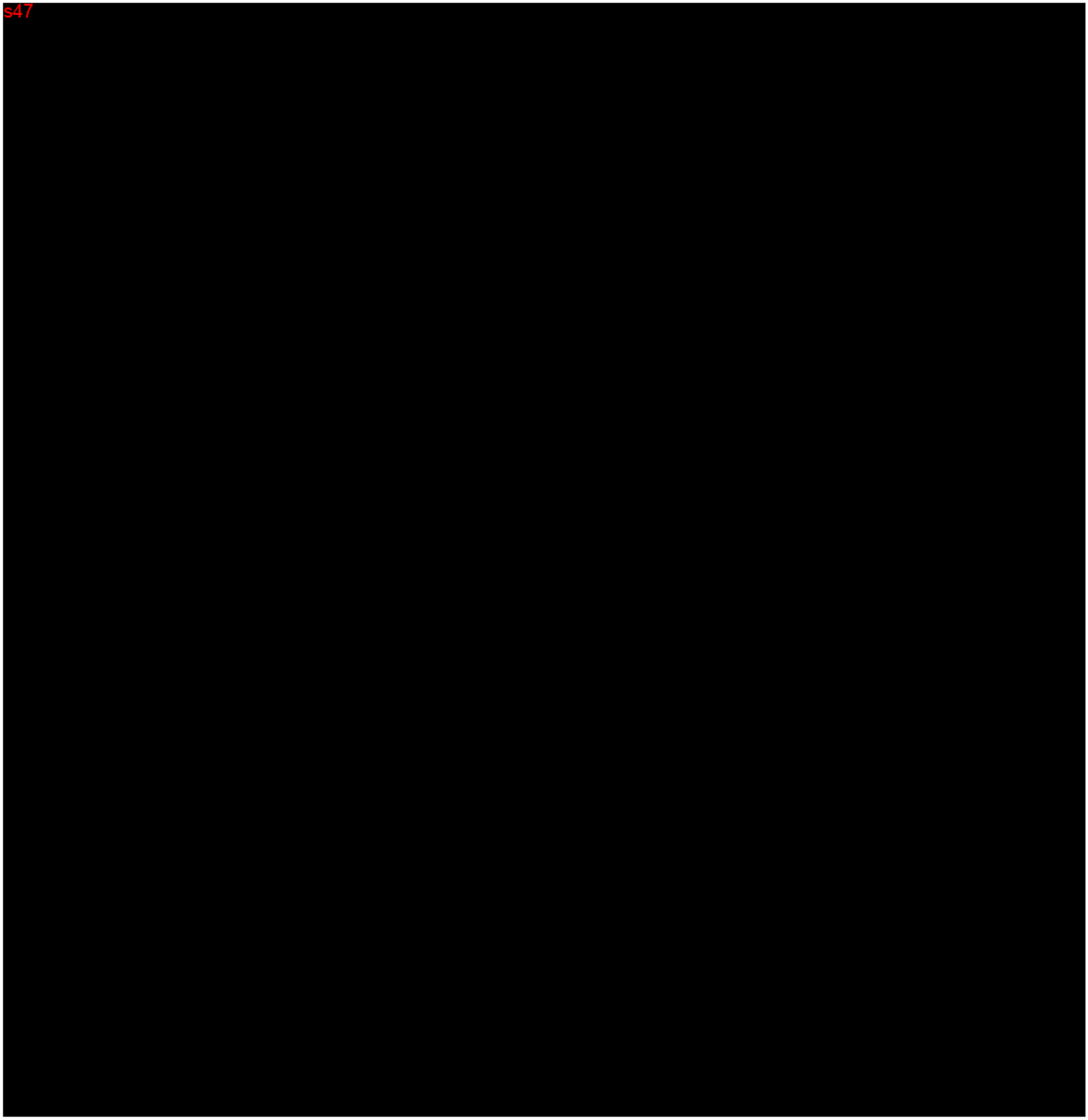
Curve Fit Results ▲

Curve Fit : Quadratic $y = A + Bx + Cx^2$

	Parameter	Estimated Value	Std. Error	Confidence Interval
Plot1 $R^2 = 0.997$	A	0.835	24.81	[-52.75, 54.43]
	B	1.050	0.052	[0.938, 1.161]
	C	-5.46e-5	2.05e-5	[-9.90e-5, -1.03e-5]

s47 [redacted]

s47



Standard Curve, Triton X-100

Sample	Well	Concentration	Values	MeanValue	Std.Dev.	CV%	Is0ng	ValRSD	0ngVa...	200ng...	400ngVal...
01	A3	0.000	25.684	25.947	0.372	1.433	1	0	25.947	0.000	0.000
	A4		26.210								
02	B3	200.000	185.595	185.202	0.556	0.300	0	0	0.000	185.202	0.000
	B4		184.809								
03	C3	400.000	350.205	352.568	3.342	0.948	0	0	0.000	0.000	352.568
	C4		354.931								
04	D3	800.000	700.574	700.876	0.428	0.061	0	0	0.000	0.000	0.000
	D4		701.179								
05	E3	1200.000	1059.573	1070.319	15.196	1.420	0	0	0.000	0.000	0.000
	E4		1081.064								
06	F3	1600.000	1402.470	1409.272	9.619	0.683	0	0	0.000	0.000	0.000
	F4		1416.074								
07	G3	2000.000	1748.738	1745.502	4.577	0.262	0	0	0.000	0.000	0.000
	G4		1742.265								
08	H3	2500.000	2276.823	2277.836	1.432	0.063	0	0	0.000	0.000	0.000
	H4		2278.848								

800ngVal...	1200ng...	1600ng...	2000ng...	2500ng...
0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000
700.876	0.000	0.000	0.000	0.000
0.000	1070.319	0.000	0.000	0.000
0.000	0.000	1409.272	0.000	0.000
0.000	0.000	0.000	1745.502	0.000
0.000	0.000	0.000	0.000	2277.836

Validation Check Sum by RSD = 0 MaxTXStdCve = 2277.836 MinTXStdCve = 25.947 Monotonic increase = VALID

Standard Curve, TE Buffer

Sample	Well	Concentration	Values	MeanValue	Std.Dev.	CV%	Is0ng	ValRSD	0ngValue	200n...	400ngV...	800ng...
01	A1	0.000	10.360	10.540	0.255	2.422	1	0	10.540	0.000	0.000	0.000
	A2		10.721									
02	B1	200.000	192.929	196.882	5.590	2.839	0	0	0.000	196....	0.000	0.000
	B2		200.834									
03	C1	400.000	395.488	400.162	6.610	1.652	0	0	0.000	0.000	400.162	0.000
	C2		404.836									
04	D1	800.000	799.724	811.397	16.508	2.035	0	0	0.000	0.000	0.000	811.397
	D2		823.070									
05	E1	1200.000	1185.287	1202.829	24.807	2.062	0	0	0.000	0.000	0.000	0.000
	E2		1220.370									
06	F1	1600.000	1525.366	1555.539	42.670	2.743	0	0	0.000	0.000	0.000	0.000
	F2		1585.711									
07	G1	2000.000	1757.042	1833.891	108.681	5.926	0	0	0.000	0.000	0.000	0.000
	G2		1910.740									
08	H1	2500.000	2241.177	2302.494	86.715	3.766	0	0	0.000	0.000	0.000	0.000
	H2		2363.810									

Standard Curve, TE Buffer (Contd)

1200ng...	1600n...	2000n...	2500n...
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
1202.829	0.000	0.000	0.000
0.000	1555....	0.000	0.000
0.000	0.000	1833.8...	0.000
0.000	0.000	0.000	2302.4...

Validation Check Sum by RSD = 0 MaxTEStdCve = 2302.494 MinTEStdCve = 10.540 Monotonic Increase = VALID

Australian Government
Department of Health and Aged Care
Therapeutic Goods Administration

Laboratories Branch

Owner: s22	**Number:** Chem-Form-62
Author: s22	**Version:** 1
Active: 15/06/2021	**Review:** 15/12/2022
Title: Assay for Lipids in Vaccines by HPLC CAD (Bio-BPC-Method-24 Pfizer)	

TGA SAMPLE No. 2211003540 **SUBSTANCES ASSAYED: ALC-0159, Cholesterol,**

Lot number: GD6794 **ALC-0315, DSPC**

METHOD REFERENCE: TM100010322 (Pfizer)/~~BIO BPC Method 24~~ Chem-Method-54 s22 1/12/22

INSTRUMENT OR SYSTEM No. 20 with CAD detector… Method Modifications approved by: s22 1/12/22

SYSTEM SUITABILITY REQUIREMENTS MET (Y/~~N~~) ASSAY REQUIREMENTS MET (Y/~~N~~)

RESULTS Strikethrough to specification made by s22 1/12/22 (Specification for bivalent formulation) s22 1/12/22

TGA Sample number	Preparation	Lipid content (mg/mL)			
		ALC-0159 s47	Cholesterol s47	ALC-0315 s47	DSPC s47
2211003540 Corrections made to include purity s22 01/12/2022	1	s47			
	2				
	3				
Average	-				
% RSD (Requirement NMT 10%)	-	1.4	0.4	0.3	0.3

ATTACHMENTS: *Analysts Notes worksheets* ☑ *Result summary report* ☑ *System suitability report* ☑ *Example chromatograms* ☑ *Run and method summary* ☑ Calibration plots ☑ MP preparation details ☑ or refer to sample ~~D22-6185836~~ D22-6187812 s22 01/12/22 D22-6180929 and D22-6186482 Other ☐……

REQUIREMENTS (Manuf.) ~~ALC-0159 s47 mg/mL; Cholesterol s47 mg/mL; ALC-0315 s47 s47 mg/mL;DSPC s47 mg/mL~~ ALC0159 s47 mg/mL; cholesterol s47 mg/mL; ALC-0315 s47 mg/mL; DSPC s47 mg/mL s22 1/12/22 Correct Requirement for bivalent formulation

Identification RT requirements met ? (RT ± 5% compared to SST) **(Y/~~N~~)**

IDENTIFIED AS ALC-0159, Cholesterol, ALC-0315 and DSPC

RESULT **PASS** ☑ **FAIL** ☐

Signature of Analyst Signed electronically by s22 Date 01/12/2022

Checked by Official Analyst…Signed electronically by s22 ……Date……2/12/22…

WORKING STANDARD CURVE PREPARATION DETAILS

Date Stock solution prepared: 28 Nov 2022 **Prepared by:** s22 **Within expiry** Y/~~N~~

Attached ☐ **or Refer to (TRIM Record number/Sample number)** D22-6180792

	ALC-0159	Cholesterol	ALC-0315	DSPC5
~~Concentration in stock solution (mg/mL)~~ s22 01/12/2022	s47			
Corrected with purity Concentration in stock solution (mg/mL) s22 01/12/2022				

Prepared by: s22 Date prepared 28 Nov 2022 POVA LIMS: 33271

Level	Mixed Lipid Soln (uL)	MeOH (uL)	DF	Final Target conc (mg/mL)	~~Actual conc (mg/mL)~~ s22 01/12/2022	Corrected Actual conc with purity (mg/mL) s22 01/12/22
1	s47			ALC-0159	s47	
				Cholesterol		
				ALC-0315		
				DSPC		
2				ALC-0159		
				Cholesterol		
				ALC-0315		
				DSPC		
3				ALC-0159		
				Cholesterol		
				ALC-0315		
				DSPC		
4				ALC-0159		
				Cholesterol		
				ALC-0315		
				DSPC		
5				ALC-0159		
				Cholesterol		
				ALC-0315		
				DSPC		

ASSAY CONTROL PREPARATION DETAILS

Date control solution prepared: 28 Nov 2022 **Within expiry** Y/~~N~~

Attached ☑ **or Refer to (TRIM/ Record number/Sample number)** D22-6180792

DP REFERENCE MATERIAL PREPARED ~~Y~~/N s22 /12/22

LIMS number	Lot number/Expiry	Sample solution (uL)	MeOH (uL)	DF
				s

SAMPLE PREPARATION DETAILS

Date prepared: 28 Nov 2022 **Prepared by:** s22 **POVA LIMS:** **33271**

Preparation	Sample solution (uL)	MeOH (uL)	DF DF 10 added by s22 1/12/22
1	s47		
2			
3			

Methanol LCMS grade; Supplier: Fisher Lot No 205074

METHOD MODIFICATIONS

s47

Australian Government
Department of Health and Aged Care
Therapeutic Goods Administration
Laboratories Branch

Owner: s22 [redacted]	**Number:** Chem-Form-62
Author: s22 [redacted]	**Version:** 1
Active: 15/06/2021	**Review:** 15/12/2022
Title: Assay for Lipids in Vaccines by HPLC CAD (Bio-BPC-Method-24 Pfizer)	

TGA SAMPLE No. 2211003544 **SUBSTANCES ASSAYED: ALC-0159, Cholesterol,**

Lot number: GE8382 **ALC-0315, DSPC**

METHOD REFERENCE: TM100010322 (Pfizer)/~~BIO BPC Method 24~~ Chem-Method-54 s22 [redacted] /12/22

INSTRUMENT OR SYSTEM No. 20 with CAD detector… Method Modifications approved by s22 [redacted] /12/22

SYSTEM SUITABILITY REQUIREMENTS MET (Y/~~N~~) ASSAY REQUIREMENTS MET (Y/~~N~~)

RESULTS Strikethrough to specification made by s22 [redacted] 1/12/22 (Specification for bivalent formulation) s22 [redacted] 1/12/22

TGA Sample number	Preparation	Lipid content (mg/mL)			
		ALC-0159 s47 [redacted]	Cholesterol s47 [redacted]	ALC-0315 s47 [redacted]	DSPC s47 [redacted]
2211003544 Corrections made to include purity CLH 01/12/2022	1	s47 [redacted]			
	2				
	3				
Average	-				
% RSD (Requirement NMT 10%)	-	1.7	0.8	0.3	1.4

ATTACHMENTS: *Analysts Notes worksheets* ☑ *Result summary report* ☑ *System suitability report* ☑ *Example chromatograms* ☑ *Run and method summary* ☑ Calibration plots ☑ MP preparation details ☑ or refer to sample ~~D22-6185836~~ D22-6187812 s22 [redacted] 01/12/22, D22-6180929 and D22-6186482 Other ☐……

REQUIREMENTS (Manuf.) ~~ALC 0159~~ s47 [redacted] ~~mg/mL; Cholesterol~~ s47 [redacted] ~~mg/mL; ALC 0315~~ s47 [redacted] s47 [redacted] ~~mg/mL;DSPC~~ s47 [redacted] ~~mg/mL~~ ALC0159 s47 [redacted] mg/mL; cholesterol s47 [redacted] mg/mL; ALC-0315 s47 [redacted] mg/mL; DSPC s47 [redacted] mg/mL s22 [redacted] /12/22 Correct Requirement for bivalent formulation

Identification RT requirements met ? (RT ± 5% compared to SST) **(Y/~~N~~)**

IDENTIFIED AS ALC-0159, Cholesterol, ALC-0315 and DSPC

RESULT **PASS** ☑ **FAIL** ☐

Signature of Analyst Signed electronically by s22 [redacted] Date 01/12/2022

Checked by Official Analyst…Signed electronically by s22 [redacted] ….Date…2/12/22……

WORKING STANDARD CURVE PREPARATION DETAILS

Date Stock solution prepared: 28 Nov 2022 **Prepared by:** s22 **Within expiry Y/~~N~~**

Attached ☐ or Refer to (TRIM Record number/Sample number) D22-6180792

	ALC-0159	Cholesterol	ALC-0315	DSPC5
~~Concentration in stock solution (mg/mL)~~ s22 01/12/2022	s47	s47	s47	s47
Corrected with purity Concentration in stock solution (mg/mL) s22 01/12/2022	s47	s47	s47	s47

Prepared by: s22 Date prepared 28 Nov 2022 POVA LIMS: 33271

Level	Mixed Lipid Soln (uL)	MeOH (uL)	DF	Final Target conc (mg/mL)	~~Actual conc (mg/mL)~~ s22 01/12/2022	Corrected Actual conc with purity (mg/mL) s22 01/12/22
1	s47			ALC-0159	s47	
				Cholesterol		
				ALC-0315		
				DSPC		
2				ALC-0159		
				Cholesterol		
				ALC-0315		
				DSPC		
3				ALC-0159		
				Cholesterol		
				ALC-0315		
				DSPC		
4				ALC-0159		
				Cholesterol		
				ALC-0315		
				DSPC		
5				ALC-0159		
				Cholesterol		
				ALC-0315		
				DSPC		

ASSAY CONTROL PREPARATION DETAILS

Date control solution prepared: 28 Nov 2022 **Within expiry Y/~~N~~**

Attached ☑ or Refer to (TRIM/ Record number/Sample number) D22-6180792

DP REFERENCE MATERIAL PREPARED ~~Y~~/N s22 **1/12/22**

LIMS number	Lot number/Expiry	Sample solution (uL)	MeOH (uL)	DF
				s47

SAMPLE PREPARATION DETAILS

Date prepared: 28 Nov 2022 **Prepared by:** s22 **POVA LIMS:** **33271**

Preparation	Sample solution (uL)	MeOH (uL)	DF DF 10 added by s22 1/12/22
1	s47		
2			
3			

Methanol LCMS grade; Supplier: Fisher Lot No 205074

METHOD MODIFICATIONS

s47

Australian Government
Department of Health
Therapeutic Goods Administration

Laboratories Branch

Operations	Biotherapeutics Laboratories Operations Manual
Procedure	PCR – Form – Quantitation of Total and Percent Encapsulated RNA in Lipid Nanoparticles by RiboGreen Fluorescence Worksheet
Written	s22 [redacted]
Authorised	
Date issued	10 August 2021
Revision #	3

Quantitation of Total and Percent Encapsulated RNA in Lipid Nanoparticles by RiboGreen Fluorescence - Worksheet

Operator	s22 [redacted]	**Date of assay**	24/11/2022
Checked by		**TRIM link to assay data file**	el://D22-6165312?db=A7&open

Results Summary

Sample Number	LIMS#
1	s22 [redacted]
2	
3	2211003544

s47 [redacted]

Reagent records

Reagent	Manufacturer and Catalogue Number	Lot Number	Expiry	Notes
Drug Substance Reference Material	PF-07305885	00711666-0110-WRM	n/a	
Quant-iT RiboGreen RNA Reagent	Thermo Fisher, R11491	2313073	n/a	
Triton X-100 10% Solution in Water	Thermo Scientific, 85111	WF322599	-	
TE Buffer	Invitrogen, AM9849	01078095	-	

Additional notes

Standard Curve Preparation

Prepare the standard curve material using aliquotted DS RM at s47 /mL, diluted according to the table below

Standard ID	In-Plate RNA Concentration (ng/mL)	Volume of DS RM at s47 mL (µL)	Volume of TE buffer (µL)
STD 1	s47		
STD 2			
STD 3			
STD 4			
STD 5			
STD 6			
STD 7			
STD 8			

Drug product sample preparation - Pfizer Comirnaty

s47

For paediatric presentation drug product, DP diluted as below;
40 uL DP + 360 uL TE buffer

Dilution factor: 62.5
Dilution factor: 250

s22

s47

Buffer Formulations

1% Triton X-100

Component	Volume Added
10% Triton X-100	s47
TE Buffer	

2% Triton X-100

Component	Volume Added
10% Triton X-100	s47
TE Buffer	

Quant-IT Ribogreen Working Solution

Component	Volume Added
Quant-IT Ribogreen Reagent	s47
TE Buffer	

Pipettes used for assay

32892, 32837, 32792, 33016

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	50µL **STD 8** 50µL TE		50µL **STD 8** 50µL 2% Triton									
B	50µL **STD 7** 50µL TE		50µL **STD 7** 50µL 2% Triton		100µL **Sample 1** in **TE** buffer	100µL **Sample 2** in **TE** buffer	100µL **Sample 3** in TE buffer					
C	50µL **STD 6** 50µL TE		50µL **STD 6** 50µL 2% Triton									
D	50µL **STD 5** 50µL TE		50µL **STD 5** 50µL 2% Triton									
E	50µL **STD 4** 50µL TE		50µL **STD 4** 50µL 2% Triton		100µL **Sample 1** in **1% Triton** buffer	100µL **Sample 2** in **1% Triton** buffer	100µL **Sample 3** in **1% Triton** buffer					
F	50µL **STD 3** 50µL TE		50µL **STD 3** 50µL 2% Triton									
G	50µL **STD 2** 50µL TE		50µL **STD 2** 50µL 2% Triton									
H	50µL **STD 1** 50µL TE		50µL **STD 1** 50µL 2% Triton									

Plus 100µL of Ribogreen working solution to each assay well

After completing the assay, convert the above to .pdf and combine it with the .pdf report generated from the SoftmaxPro assay data file to form a single file containing both the worksheet and results. File the combined .pdf and the raw softmax data (.sda) in the assay specific data folder (E21-207399)

s47

Results Summary

Results Summary **LIMS# 33237** **Version 1, Feb2021** **Validated 01/02/22 - Next Due 01/02/23**
Pfizer - BNT162b2
Quantitation of Total and Percent Encapsulated RNA in PF-07302048 Lipid Nanoparticles by RiboGreen Fluorescence

Assay Validity Criteria		Observed Value	Validity
Coefficient of determination (R^2) for TE standard curve	≥ 0.98	= 0.997	VALID
Coefficient of determination (R^2) for TX standard curve	≥ 0.98	= 0.999	VALID
%RSD for all standard curve points (point 8, 0ng/mL, excluded)	≤ 20%	Pass	VALID
Monotonous increase over TE standard curve	Present	Present	VALID
Monotonous increase over TX standard curve	Present	Present	VALID

s47 [redacted]

Sample Validity Criteria:

s47 [redacted]

s47 [redacted]

Spreadsheet validation procedure:

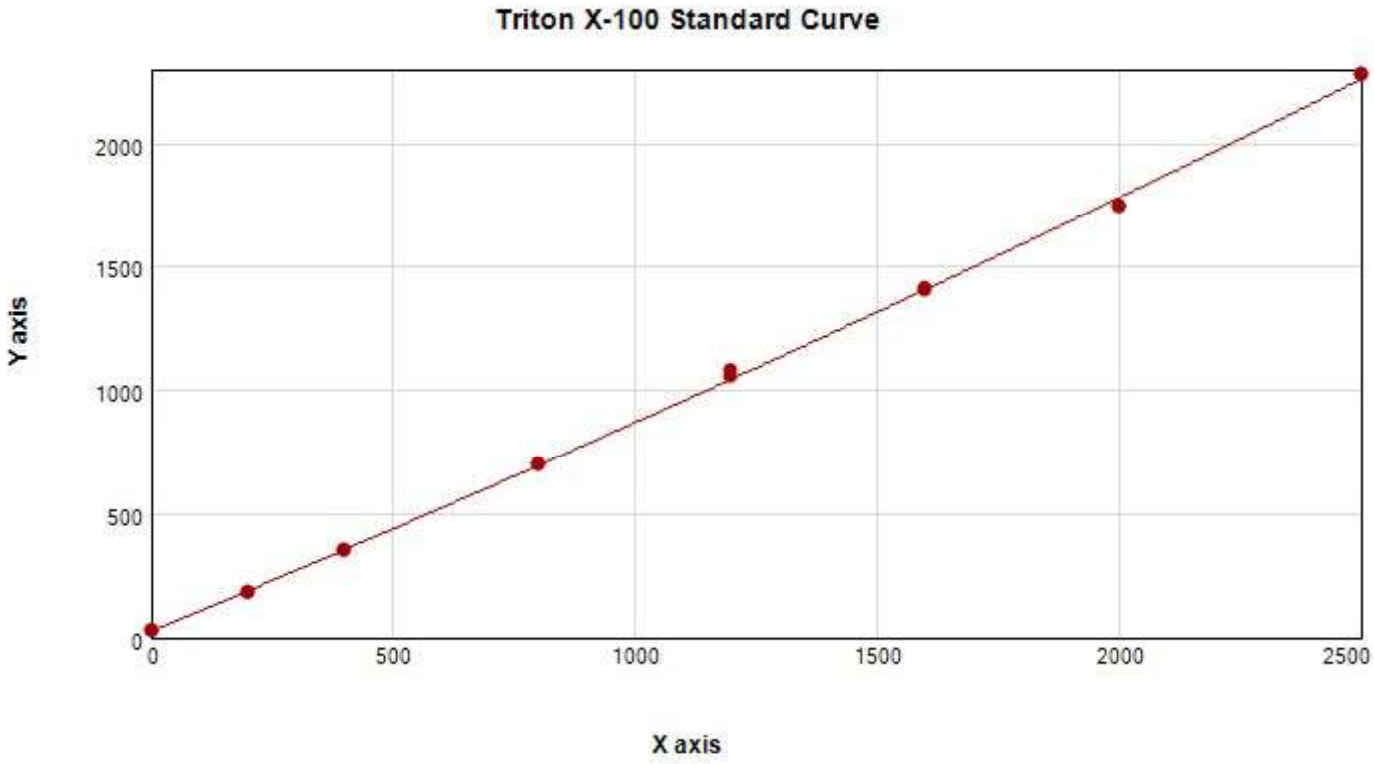
Open the active copy of Softmax Pro template "Quantitation of Total and Percent Encapsulated RNA in PF-07302048 Lipid Nanoparticles by RiboGreen Fluorescence.spr".

Open spreadsheet validation data set (TRIM link: D21-2134364) and copy well data from Plate 1. Paste well data into Plate 1 in the Softmax Pro Template undergonig validation.

Generate a .pdf printout. File the .pdf in the validation folder in TRIM (E21-212109), using thefile name "Spreadsheet validation results - LIMS#33237 - DDMMMYY - username.pdf"

If the calculated values and validity status are consistent between the generated report and those recorded in the validation dataset report (TRIM link: D21-2134332) the template is considered valid, and a "PASS" result is to be entered into qLIMS. Update the validation date and due date in the template and submit a zipped version of the template for storage in q-pulse.

In the event of inconsistent results, a "FAIL" result is to be enetered into qLIMS and the issue is to be reported to the PCR Unit Manager.

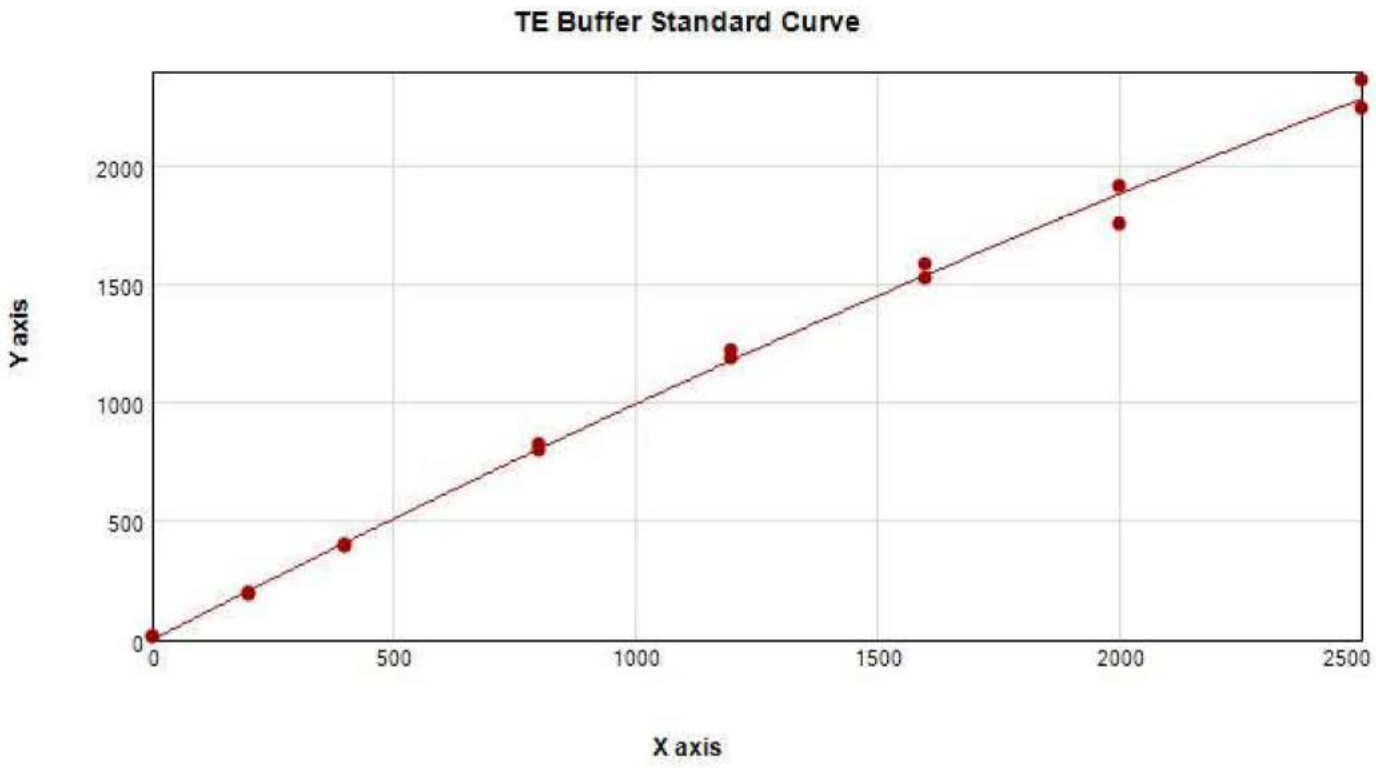


● **Plot2** (Standard Curve, Triton X-100: Values vs Concentration) Weighting: Fixed

Curve Fit Results ▲

Curve Fit : Quadratic $y = A + Bx + Cx^2$

	Parameter	Estimated Value	Std. Error	Confidence Interval
Plot2 $R^2 = 0.999$	A	26.04	9.793	[4.884, 47.20]
	B	0.812	0.020	[0.768, 0.856]
	C	3.26e-5	8.11e-6	[1.51e-5, 5.01e-5]



● **Plot1** (Standard Curve, TE Buffer: Values vs Concentration) Weighting: Fixed

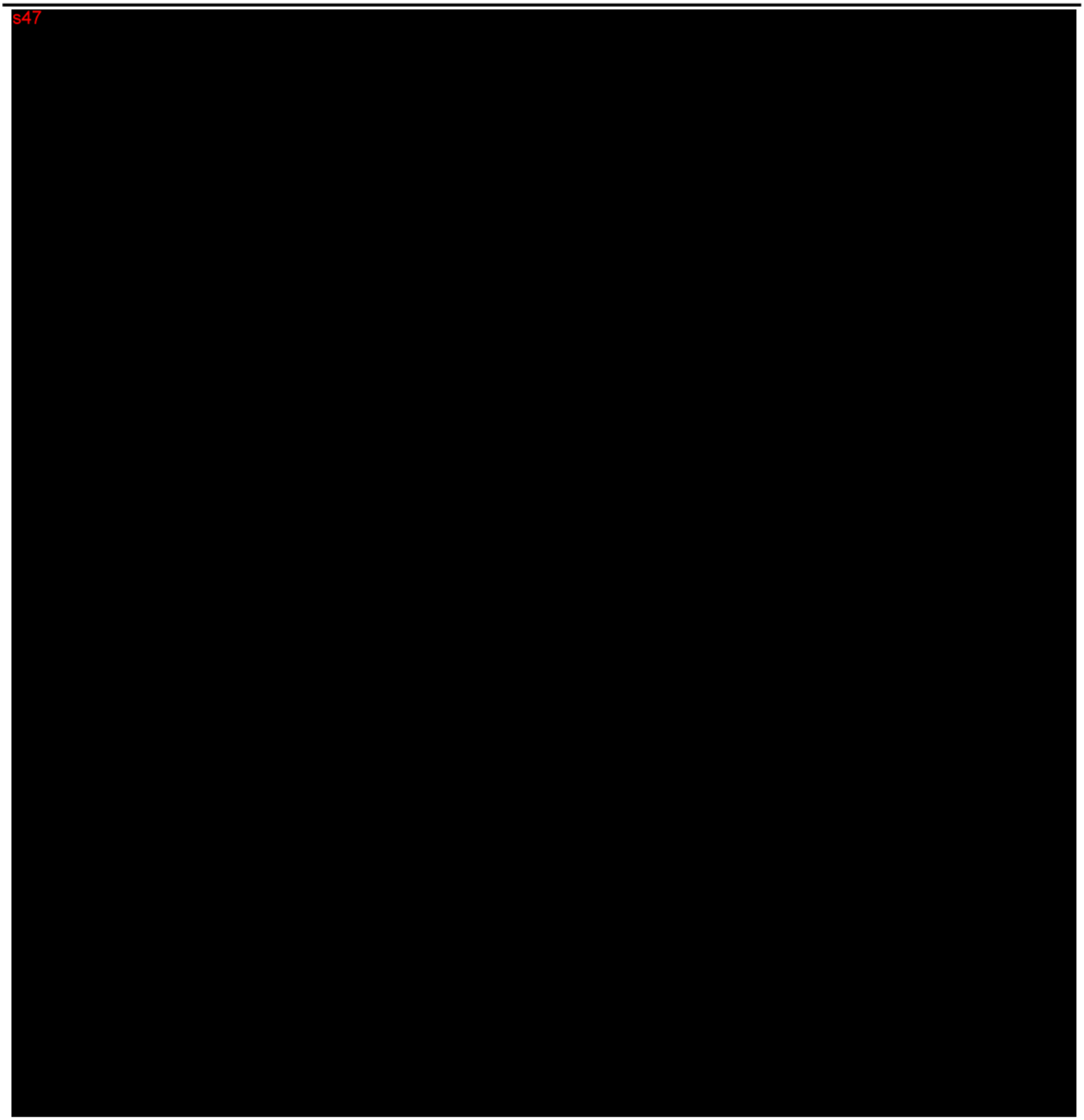
Curve Fit Results ▲

Curve Fit : Quadratic $y = A + Bx + Cx^2$

	Parameter	Estimated Value	Std. Error	Confidence Interval
Plot1 $R^2 = 0.997$	A	0.835	24.81	[-52.75, 54.43]
	B	1.050	0.052	[0.938, 1.161]
	C	-5.46e-5	2.05e-5	[-9.90e-5, -1.03e-5]

s47 [redacted]

s47



Standard Curve, Triton X-100

Sample	Well	Concentration	Values	MeanValue	Std.Dev.	CV%	Is0ng	ValRSD	0ngVa...	200ng...	400ngVal...
01	A3	0.000	25.684	25.947	0.372	1.433	1	0	25.947	0.000	0.000
	A4		26.210								
02	B3	200.000	185.595	185.202	0.556	0.300	0	0	0.000	185.202	0.000
	B4		184.809								
03	C3	400.000	350.205	352.568	3.342	0.948	0	0	0.000	0.000	352.568
	C4		354.931								
04	D3	800.000	700.574	700.876	0.428	0.061	0	0	0.000	0.000	0.000
	D4		701.179								
05	E3	1200.000	1059.573	1070.319	15.196	1.420	0	0	0.000	0.000	0.000
	E4		1081.064								
06	F3	1600.000	1402.470	1409.272	9.619	0.683	0	0	0.000	0.000	0.000
	F4		1416.074								
07	G3	2000.000	1748.738	1745.502	4.577	0.262	0	0	0.000	0.000	0.000
	G4		1742.265								
08	H3	2500.000	2276.823	2277.836	1.432	0.063	0	0	0.000	0.000	0.000
	H4		2278.848								

800ngVal...	1200ng...	1600ng...	2000ng...	2500ng...
0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000
700.876	0.000	0.000	0.000	0.000
0.000	1070.319	0.000	0.000	0.000
0.000	0.000	1409.272	0.000	0.000
0.000	0.000	0.000	1745.502	0.000
0.000	0.000	0.000	0.000	2277.836

Validation Check Sum by RSD = 0 MaxTXStdCve = 2277.836 MinTXStdCve = 25.947 Monotonic increase = VALID

Standard Curve, TE Buffer

Sample	Well	Concentration	Values	MeanValue	Std.Dev.	CV%	Is0ng	ValRSD	0ngValue	200n...	400ngV...	800ng...
01	A1	0.000	10.360	10.540	0.255	2.422	1	0	10.540	0.000	0.000	0.000
	A2		10.721									
02	B1	200.000	192.929	196.882	5.590	2.839	0	0	0.000	196....	0.000	0.000
	B2		200.834									
03	C1	400.000	395.488	400.162	6.610	1.652	0	0	0.000	0.000	400.162	0.000
	C2		404.836									
04	D1	800.000	799.724	811.397	16.508	2.035	0	0	0.000	0.000	0.000	811.397
	D2		823.070									
05	E1	1200.000	1185.287	1202.829	24.807	2.062	0	0	0.000	0.000	0.000	0.000
	E2		1220.370									
06	F1	1600.000	1525.366	1555.539	42.670	2.743	0	0	0.000	0.000	0.000	0.000
	F2		1585.711									
07	G1	2000.000	1757.042	1833.891	108.681	5.926	0	0	0.000	0.000	0.000	0.000
	G2		1910.740									
08	H1	2500.000	2241.177	2302.494	86.715	3.766	0	0	0.000	0.000	0.000	0.000
	H2		2363.810									

Standard Curve, TE Buffer (Contd)

1200ng...	1600n...	2000n...	2500n...
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
1202.829	0.000	0.000	0.000
0.000	1555....	0.000	0.000
0.000	0.000	1833.8...	0.000
0.000	0.000	0.000	2302.4...

Validation Check Sum by RSD = 0 MaxTEStdCve = 2302.494 MinTEStdCve = 10.540 Monotonic Increase = VALID

TABLE OF CONTENTS

LIST OF TABLES

3.2.P.8.1. STABILITY SUMMARY AND CONCLUSION –BIVALENT (ORIGINAL AND OMICRON (B.1.1.529)) DRUG PRODUCT

3.2.P.8.1.1. Drug Product Shelf Life at Recommended Storage Temperature

The shelf-life of the BNT162b2 Bivalent [Original and Omicron (B.1.1.529)] (herein referred to as Bivalent) drug product is 18 months. Refer to Section 3.2.P.8.3 Stability Data – Long-Term Storage – Tris-Sucrose for additional stability data to support the shelf-life when stored at the intended storage condition of -90 to -60 °C. The shelf-life is the same as on the shelf-life for the Original drug product (Tris/Sucrose formulation). The shelf-life also includes an allowance for short-term storage at 5 ± 3°C for up to 10 weeks (not exceeding expiry).

Bivalent drug product lots are enrolled in the stability programs are being monitored in accordance with the stability protocols outlined below to confirm the shelf-life when stored at the intended storage condition of -90 to -60 °C. All testing to date has been performed using analytical methodology and phase appropriate specifications in place at time of testing. The analytical procedures used in the stability programs were developed to monitor the composition, strength, purity, safety and general quality attributes of the drug product.

3.2.P.8.1.2. Bivalent Stability Batches and Studies

One Bivalent drug product confirmatory stability batch and one additional supportive stability batch are enrolled in stability programs and monitored in accordance with the approved protocols. The stability program is designed to follow ICH guidelines for stability of drug product (ICH Guideline Q1A: Stability Testing of New Drug Substances and Products; ICH Guideline Q5C: Quality of Biotechnological Products, Stability Testing of Biotechnological/Biological Products). A summary of all drug product lots on stability studies and current available stability data are shown in Table 3.2.P.8.1-1.

Table 3.2.P.8.1-1. Summary of On-going Stability Studies

Lot Number	Drug Product Batch Use	Stability Study Start	Study Type	Storage Condition	Data Available	Study Status
22-DP-01012 (Pfizer Andover)	Stability, Clinical	March 2022	Long Term	-90 to -60 °C	6 months	Ongoing
			Accelerated	-20 ± 5 °C	1 month	Complete
			Accelerated	5 ± 3 °C	6 months	Complete
GA2789 (Pfizer Puurs)	Large Scale Confirmatory, Stability	March 2022	Long Term	-90 to -60 °C	6 months	Ongoing
			Accelerated	-20 ± 5 °C	1 month	Complete
			Accelerated	5 ± 3 °C	3 months	Complete

3.2.P.8.1.3. Analytical Procedures Used in the Bivalent Stability Monitoring Program

The quality of drug product lots placed in the stability program is monitored using multiple analytical tests, each with pre-established acceptance criteria. The analytical tests used in the

stability monitoring program were developed to monitor composition, strength, purity, safety and general quality attributes of the drug product. The analytical procedures used in the stability-monitoring program during clinical development and the commercial stability procedures are listed in Section 3.2.P.5.2 Analytical Procedures - Overview - Bivalent [Original and Omicron (B.1.1.529)].

3.2.P.8.1.4. Bivalent Stability Program Specifications

The data obtained during the stability program were evaluated against the clinical stability specifications and the commercial stability specifications where appropriate. The commercial specification(s) (release and shelf life) are provided in Section 3.2.P.5.1 Specifications - Bivalent [Original and Omicron (B.1.1.529)].

3.2.P.8.1.5. Summary of the Bivalent Stability Program

Bivalent drug product lots used in the stability studies were packaged in glass vials, which are either the commercial packaging or the same as the commercial packaging. Vials were stored upright (cap-up) unless otherwise noted.

3.2.P.8.1.5.1. Protocol for Testing at the Long Term Condition (-90 to -60 °C)

Vials from Bivalent drug product lots are stored at the recommended storage condition of -90 to -60 °C. Testing is performed according to the protocol indicated in Table 3.2.P.8.1-2 for the Bivalent CTM drug product lot 22-DP-0102 and in Table 3.2.P.8.1-3 for the Bivalent large-scale confirmatory drug product lot GA2789.

Table 3.2.P.8.1-2. Protocol for Bivalent CTM DP Lot 22-DP-0102 at the Long-Term Condition of -90 to -60°C

Analytical Procedure	Test Interval [a]
Appearance (Visible)	0, 1M, 2M, 3M, 6M, 9M, 12M, 18M, 24M
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 6M, 12M, 18M, 24M
Container Closure Integrity Test	12M, 24M
Endotoxin	0, 12M, 24M
Sterility	

a. Initial data (t0) are from release testing.
W = weeks; M = months; LNP = Lipid Nanoparticle

Table 3.2.P.8.1-3. Protocol for Bivalent Large Scale Confirmatory DP Lot GA2789 at the Long-Term Condition of -90 to -60°C

Analytical Procedure	Test Interval [a]
Appearance (Visible)	0, 1M, 2M, 3M, 6M, 12M, 18M, 24M
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 6M, 12M, 18M, 24M
Container Closure Integrity Test	0, 12M, 24M
Endotoxin	
Sterility	

a. Initial data (t0) are from release testing.
M = months; LNP = Lipid Nanoparticle

3.2.P.8.1.5.2. Protocol for Testing at the Accelerated Condition

Bivalent drug product lots were stored under the accelerated condition of -20 ± 5 °C and 5 ± 3 °C. Testing at -20 ± 5 °C is performed according to the protocol indicated in Table 3.2.P.8.1-4 for the Bivalent CTM drug product lot 22-DP-0102 and in Table 3.2.P.8.1-5 for the Bivalent large-scale confirmatory drug product lot GA2789.

Table 3.2.P.8.1-4. Protocol for Bivalent CTM Lot 22-DP-01012 at the Accelerated Condition of -20 ± 5°C

Analytical Procedure	Test Interval [a]
Appearance (Visible)	0, 2W, 1M
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	

a. Initial data (t0) are from release testing.
W = weeks; M = months; LNP = Lipid Nanoparticle

Table 3.2.P.8.1-5. Protocol for Bivalent Large Scale Confirmatory DP Lot GA2789 at the Accelerated Condition of -20 ± 5°C

Analytical Procedure	Test Interval [a]
Appearance (Visible)	0, 1M
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	

a. Initial data (t0) are from release testing.
W = weeks; M = months; LNP = Lipid Nanoparticle

Testing at 5 ± 3 °C is performed according to the protocol indicated in Table 3.2.P.8.1-6 for Bivalent CTM drug product lot 22-DP-01012 and in Table 3.2.P.8.1-7 for Bivalent large-scale confirmatory lot GA2789.

Table 3.2.P.8.1-6. Protocol for Bivalent CTM DP Lot 22-DP-01012 at the Accelerated Condition of 5 ± 3°C

Analytical Procedure	Test Interval [a]
Appearance (Visible)	0, 1M, 3M, 4M, 6M
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 6M

a. Initial data (t0) are from release testing.
M = months; LNP = Lipid Nanoparticle

Table 3.2.P.8.1-7. Protocol for Bivalent Large Scale Confirmatory DP Lot GA2789 at the Accelerated Condition of 5 ± 3°C

Analytical Procedure	Test Interval [a]
Appearance (Visible)	0, 1M, 3M
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 3M

a. Initial data (t0) are from release testing.
M = months; LNP = Lipid Nanoparticle

3.2.P.8.1.6. Summary of Bivalent Stability Data

3.2.P.8.1.6.1. Summary of Bivalent Stability Data at the Long Term Storage Condition (-90 to -60 °C)

Results from a CTM and a confirmatory batch stability study on Bivalent drug product stored at the long-term condition of -90 to -60°C are provided in Section 3.2 P.8.3 Stability Data Long-Term - Bivalent [Original and Omicron (B.1.1.529)].

Six months of data are available for Bivalent CTM drug product. All data remained within the clinical acceptance criteria and meet the commercial acceptance criteria.

Six months of data is available for Bivalent large scale confirmatory drug product batch GA2789. All data met the acceptance criteria.

Overall, the data indicate that there have been no significant changes in terms of quality, purity, or strength for the drug product.

3.2.P.8.1.6.2. Summary of Stability Data at the Accelerated Storage Condition

Results from a CTM and a confirmatory batch stability study on Bivalent drug product stored at the accelerated conditions of -20 ± 5°C and 5 ± 3°C are provided in Section 3.2.P.8.3 Stability Data Additional Storage Condition - Bivalent [Original and Omicron (B.1.1.529)].

Accelerated -20 ± 5 °C Stability

One (1) month of data are available for Bivalent CTM drug product. All data remained within the clinical acceptance criteria and meet the commercial acceptance criteria.

One (1) month of data is available for BNT162b2 large scale confirmatory drug product batch GA2789. All data met the acceptance criteria.

Overall, the data indicate that there have been no significant changes in terms of quality, purity, or strength for the drug product. This study is complete at 1 month.

Accelerated 5 ± 3 °C Stability

Six months of data are available for Bivalent CTM drug product. All data remained within the clinical acceptance criteria and meet the commercial acceptance criteria through 4 months. LNP polydispersity does not meet clinical or commercial acceptance criteria at 6 months.

Three months of data is available for BNT162b2 large scale confirmatory drug product batch GA2789. All data met the acceptance criteria.

Overall, the data indicate that there have been no significant changes in terms of quality, purity, or strength for the drug product and support short term storage at 5 ± 3 °C for up to 10 weeks.

3.2.P.8.1.7. Shelf Life and Conclusions

The shelf-life for the BNT162b2 Bivalent (Original and Omicron (B.1.1.529)) drug product is 18 months when stored at the recommended temperature of -90 to -60 °C, including short-term storage at 5 ± 3°C for up to 10 weeks (within the 18-month shelf-life).

The shelf-life is based on:

- The shelf-life for the Original Tris/Sucrose drug product, which is based on stability data obtained at the intended storage condition (-90 to -60 °C) as well as the accelerated storage conditions (-20 ± 5°C and 5 ± 3°C) during primary stability studies.

- Up to 6 months of stability data for Bivalent drug product clinical and confirmatory batches at the intended storage condition (-90 to -60 °C) as well as the accelerated storage conditions (-20 ± 5°C and the 5 ± 3°C).

These stability studies are currently on-going and data from these studies will be used to confirm the shelf shelf-life of the Bivalent drug product. The Original tris/sucrose studies are also on-going and will be used to extend the shelf life based on the acceptability of the data.

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3.2.P.8.3. STABILITY DATA

Data from confirmatory and supportive stability studies on BNT162b2 Bivalent [Original and Omicron (B.1.1.529)] (herein referred to as Bivalent) drug product lots stored at the long-term condition of -90 to -60 °C are presented.

All studies are listed in Table 3.2.P.8.3-1. Results are provided in Table 3.2.P.8.3-2 through Table 3.2.P.8.3-3.

Stability testing was performed according to the acceptance criteria in place at the time of testing. The acceptance criteria provided in the tables below represent those in place where the latest time-point tested.

Table 3.2.P.8.3-1. Summary Table of Bivalent Drug Product Lots

Lot Number	Drug Product Lot Use	Stability Study Start	Stability Data Presented	Table Location
22-DP-01012	Stability, clinical	March 2022	-90 to -60 °C: 6 months	Table 3.2.P.8.3-2
GA2789	Large Scale Confirmatory, Stability	March 2022	-90 to -60 °C: 6 months	Table 3.2.P.8.3-3

Table 3.2.P.8.3-2. Stability Data for Bivalent Drug Product Lot 22-DP-01012 Stored at -90 to -60 °C

Analytical Procedure/Quality Attribute	Appearance		pH	Subvisible Particles	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visible)	Visible Particulates			LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content
Timepoint/ Acceptance Criteria	**White to off-white suspension**	**May contain white to off-white opaque, amorphous particles**	**7.4 ± 0.5**	**≥10µm: ≤6000/container ≥25µm: ≤600/container**	s47			
0	WOS	Meets (EFVP)	7.3	s47				
1M	WOS	Meets (EFVP)	7.2					
2M	WOS	Meets (EFVP)	7.3					
3M	WOS	Meets (EFVP)	7.4					
6M	WOS	Meets (EFVP)	7.4					
9M	S	S	S	NS	S	S	S	S
12M	S	S	S	S	S	S	S	S
18M	S	S	S	S	S	S	S	S
24M	S	S	S	S	S	S	S	S

M = Month, S = To be Scheduled, NS = Not Scheduled at Time Point, LNP = Lipid Nanoparticle; WOS = White to off-white suspension, EFVP = Essentially free from visible particulates, NGD = No growth detected

Table 3.2.P.8.3-2. Stability Data for Bivalent Drug Product Lot 22-DP-01012 Stored at -90 to -60 °C

Analytical Procedure/Quality Attribute	HPLC-CAD				Cell-based Flow Cytometry	Capillary Gel Electrophoresis	Endotoxin (LAL)	Sterility	Dye Incursion
	ALC-0315 Content	ALC-0159 Content	DSPC Content	Cholesterol Content	In Vitro Expression	RNA Integrity			Container Closure Integrity
Timepoint/ Acceptance Criteria	s47				Report Results (% cells positive)	s47	s47	No growth detected	Pass
0	s47						s47	NGD	NS
1M							NS	NS	NS
2M							NS	NS	NS
3M							NS	NS	NS
6M							NS	NS	NS
9M	S	S	S	S	S	S	NS	NS	NS
12M	S	S	S	S	S	S	S	S	S
18M	S	S	S	S	S	S	NS	NS	NS
24M	S	S	S	S	S	S	S	S	S

M = Month, S = To be Scheduled, NS = Not Scheduled at Time Point, LNP = Lipid Nanoparticle; WOS = White to off-white suspension, EFVP = Essentially free from visible particulates, NGD = No growth detected

Table 3.2.P.8.3-3. Stability Data for Bivalent Drug Product Lot GA2789 Stored at -90 to -60 °C

Analytical Procedure/Quality Attribute	Appearance		pH	Subvisible Particles	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visible)	Visible Particulates			LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content
Timepoint/ Acceptance Criteria	White to off-white suspension	May contain white to off-white opaque, amorphous particles	7.4 ± 0.5	≥10µm: ≤6000/container ≥25µm: ≤600/container	s47			
0[a]	Meets	Meets	7.4	s47				
1M	Meets	Meets	7.5					
2M	Meets	Meets	7.5					
3M	Meets	Meets	7.4					
6M	Meets	Meets	7.5					
12M	S	S	S	S	S	S	S	S
18M	S	S	S	S	S	S	S	S
24M	S	S	S	S	S	S	S	S

Analytical Procedure/Quality Attribute	HPLC-CAD				Cell-based Flow Cytometry	Capillary Gel Electrophoresis	Endotoxin (LAL)	Sterility	Dye Incursion
	ALC-0315 Content	ALC-0159 Content	DSPC Content	Cholesterol Content	In Vitro Expression	RNA Integrity			Container Closure Integrity
Timepoint/ Acceptance Criteria	s47				s47	s47	s47	No growth detected	Pass
0[a]	s47						s47	NGD	Pass
1M							NS	NS	NS
2M							NS	NS	NS
3M							NS	NS	NS
6M							NS	NS	NS
12M	S	S	S	S	S	S	S	S	S
18M	S	S	S	S	S	S	NS	NS	NS
24M	S	S	S	S	S	S	S	S	S

a. Analysis for T0 was repeated for this study. Initial data (T0) of lot GA2789 is not from release testing, with the exception of sterility, endotoxin and particulate matter.

M = Month, S = To be Scheduled, NS = Not Scheduled at Time Point, LNP = Lipid Nanoparticle; NGD = No growth detected

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3.2.P.8.1. STABILITY SUMMARY AND CONCLUSION – TRIS/SUCROSE DRUG PRODUCT

The commercial shelf life of the BNT162b2 Tris/Sucrose drug product is 18 months when stored at the intended storage condition of -90 to -60 °C. This shelf life is based on 18 months stability data from three BNT162b2 Tris/Sucrose primary drug product lots and 24 weeks Tris/Sucrose development stability data. The stability data generated for BNT162b2 Tris/Sucrose drug product lots and the Puurs PPQ lots also support the additional storage conditions of 5 ± 3 °C for up to 3 months, within the 18-month shelf-life, supporting the subsequent storage conditions of 2-8 °C for 10 weeks at the point of use.

Drug product lots enrolled in the stability programs are being monitored in accordance with the approved stability protocols. All testing to date has been performed using the analytical methodology and specifications in place at time of testing. The analytical procedures used in the stability programs were developed to monitor the composition, strength, purity, safety and general quality attributes of the drug product.

3.2.P.8.1.1. Drug Product Shelf Life at the Long-Term Storage Temperature of -90 to -60 °C

The shelf life of the BNT162b2 Tris/Sucrose drug product is 18 months when stored at the long-term storage condition of -90 to -60 °C. The shelf life claim is based on 18 months stability data for three BNT162b2 Tris/Sucrose primary drug product lots stored at -90 to -60 °C, up to 12 months stability data from the PPQ lots manufactured at Puurs and24 weeks Tris/Sucrose development stability data.

Additionally, the stability data generated to date at 5 ± 3 °C for the BNT162b2 Tris/Sucrose primary drug product lots and the Puurs PPQ lots also support the additional storage condition at 5 ± 3 °C for up to 3 months (within the 18 month shelf life).

3.2.P.8.1.2. In-use Period of Drug Product

Stability data and compatibility studies supporting the in-use period of the BNT162b2 Tris/Sucrose drug product at the administration site is provided in Section 3.2.P.2.6 Compatibility – Tris-Sucrose.

3.2.P.8.1.3. Stability Batches and Studies

The stability program is designed to follow ICH guidelines for stability of drug product (ICH Guideline Q1A: Stability Testing of New Drug Substances and Products; ICH Guideline Q5C: Quality of Biotechnological Products, Stability Testing of Biotechnological/Biological Products). The drug product lots placed on stability were packaged in the glass vials to be used for commercial packaging. A summary of the Tris/Sucrose drug product lots on stability and current available stability data are provided in Table 3.2.P.8.1-1.

3.2.P.8.1.3.1. Primary Drug Product Lots

Three BNT162b2 Tris/Sucrose primary drug product lots have been placed under long term storage conditions, additional storage conditions and thermal stress and thermal cycling conditions. These primary drug product lots, EX0490, EW4564 and EW4565, were manufactured at Pfizer, Puurs as detailed in Section 3.2.P.2.3 Development History –

Tris-Sucrose. The genealogies of these lots are provided in Section 3.2.P.2.3 Lot Genealogy and Usage – Tris-Sucrose.

For each of the three primary drug product lots, the drug substances and LNPs through LNP formation and stabilization were manufactured at 139 g commercial scale using the PBS/Sucrose commercial manufacturing processes, which are unchanged for the Tris/Sucrose formulation. Twelve gram quantities of the LNPs were then further processed by buffer exchange into Tris buffer, concentration adjustment, and addition of sucrose. A portion of this Tris/Sucrose bulk drug product was used for fill and finish. The 12 g scale of manufacture for these primary drug product lots was approximately 7% of the planned commercial scale manufacture of 160 grams of RNA. These primary drug product lots are representative of the commercial Tris/Sucrose drug product lots.

Lot EX0490 was filled at a volume of 0.48 mL per vial to provide a single dose of 30 µg RNA in 0.3 mL injection volume. Lots EW4564 and EW4565 were filled at a volume of 2.25 mL per vial to provide 6 doses of 30 µg RNA in 0.3 mL injection volume. Based upon current understanding of the product characteristics, drug product manufacturing process and ICH Guideline: Q1D Bracketing and Matrix Designs for Stability Testing of New Drug Substance and Products, concepts and principles, all three lots provide support for shelf life establishment of both the single dose and multidose vial.

A summary of the stability data obtained with these three primary drug product lots is provided in Section 3.2.P.8.1.9.

3.2.P.8.1.3.2. Puurs PPQ Lots Filled at 2.25 mL Fill Volume

Three Puurs PPQ lots are being evaluated for stability under long term and additional storage conditions. These lots, FC8273, FE4394 and FJ5683, were manufactured at 1600 L batch scale and filled into vials at 2.25 mL fill volume, as detailed in Section 3.2.P.3.5 LNP Formation and Drug Product Formulation and Fill - Tris-Sucrose [Puurs].

A Summary of the Tris/Sucrose drug product lots, filled at 2.25 fill volume, on stability and current available stability data are shown in Table 3.2.P.8.1-1.

3.2.P.8.1.3.3. Puurs PPQ Lots Filled at 1.3 and 0.4 mL Fill Volumes

Three Puurs PPQ lots, one of which was split into two distinct fill lots, are being evaluated for stability under long term and additional storage conditions. These lots, FK5127, FK5128, FK5618, and FM0703 were manufactured at 1600 L batch scale and filled on WSL10. The first PPQ lot was split into 1550 L and 50 L portions and filled into vials at 1.3 mL, as lot FK5127 and 0.4 mL, as lot FK5128, respectively. The second PPQ lot was filled at 1.3 mL as lot FK5618, as detailed in Section 3.2.P.3.5 LNP Production Drug Product Formulation and Fill Finish – 1.3 mL and 0.4 mL Fills – Tris-Sucrose [Puurs].

Summary of the Tris/Sucrose drug product lots, filled at 1.3 and 0.4 fill volume, on stability and current available stability data are shown in Table 3.2.P.8.1-1.

3.2.P.8.1.3.4. Supporting Stability Lots

In addition to the primary stability studies for the Tris/Sucrose drug product and the initial PPQ batches supporting the 2.25, 1.3 and 0.4 mL fill volume discussed above, further lots have been included into stability studies as depicted in Table 3.2.P.8.1-1.

Table 3.2.P.8.1-1. Summary of On-going Stability Studies

Lot Number	Stability Study Start	Drug Product Batch Use	Study Type	Storage Condition	Data Available	Study Status
EX0490	March 2021	Primary Stability	Long Term	-90 to -60 °C	18 months	On-going
			Additional	-50 ± 5 °C	1 month	Complete
			Additional	-20 ± 5 °C	6 months	Complete
			Additional	5 ± 3 °C	6 months	Complete
			Thermal Stress	25 ± 2 °C/60 ± 5% RH	1 month	Complete
			Thermal Stress	30 ± 2 °C/65 ± 5% RH	1 month	Complete
			Thermal Cycling 1	1 month at -20 ± 5 °C followed by 6 months at 5 ± 3 °C.	7 months	Complete
			Thermal Cycling 2	2 months at -20 ± 5 °C followed by 3 months at 5 ± 3 °C.	5 months	Complete
			Thermal Cycling 3	3 months at -20 ± 5 °C followed by 3 months at 5 ± 3 °C.	6 months	Complete
			Thermal Cycling 4	1 cycle of 1 month at -90 to -60 °C and 1 month at -50 ± 5 °C. 2 cycles of 1 month at -20 ± 5 °C and 1 month at -90 to -60 °C.	6 months	Complete
			Thermal Cycling 5	5 cycles of 4 days at -20 ± 5 °C and 1 day at 25 ± 2 °C/60 ± 5% RH. After cycling, move to -50 ± 5 °C until 2 months then transfer to -90 to -60 °C.	12 months	On-going
	Jan 2022		Thermal Cycling 6	10 months at -90 to -60 °C and 4 months at 5±3 °C	4 months	Complete
EW4564	April 2021	Primary Stability	Long Term	-90 to -60 °C	18 months	On-going
			Additional	-50 ± 5 °C	1 month	Complete
			Additional	-20 ± 5 °C	6 months	Complete
			Additional	5 ± 3 °C	6 months	Complete
			Thermal Stress	25 ± 2 °C/60 ± 5% RH	1 month	Complete
			Thermal Stress	30 ± 2 °C/65 ± 5% RH	1 month	Complete
			Thermal Cycling 1	1 month at -20 ± 5 °C followed by 6 months at 5 ± 3 °C.	7 months	Complete
			Thermal Cycling 2	2 months at -20 ± 5 °C followed by 3 months at 5 ± 3 °C.	5 months	Complete
			Thermal Cycling 3	3 months at -20 ± 5 °C followed by 3 months at 5 ± 3 °C.	6 months	Complete
			Thermal Cycling 4	1 cycle of 1 month at -90 to -60 °C and 1 month at -50 ± 5 °C. 2 cycles of 1 month at -20 ± 5 °C and 1 month at -90 to -60 °C.	6 months	Complete

Table 3.2.P.8.1-1. Summary of On-going Stability Studies

Lot Number	Stability Study Start	Drug Product Batch Use	Study Type	Storage Condition	Data Available	Study Status
			Thermal Cycling 5	5 cycles of 4 days at -20 ± 5 °C and 1 day at 25 ± 2 °C/60 ± 5% RH. After cycling, move to -50 ± 5 °C until 2 months then transfer to -90 to -60 °C.	12 months	On-going
			Photostability	With Light Protection and Without Light Protection		Complete
EW4565	April 2021	Primary Stability	Long Term	-90 to -60 °C	18 months	On-going
			Additional	-50 ± 5 °C	1 month	Complete
			Additional	-20 ± 5 °C	6 months	Complete
			Additional	5 ± 3 °C	6 months	Complete
			Thermal Cycling 1	1 month at -20 ± 5 °C followed by 6 months at 5 ± 3 °C.	7 months	Complete
			Thermal Cycling 2	2 months at -20 ± 5 °C followed by 3 months at 5 ± 3 °C.	5 months	Complete
			Thermal Cycling 3	3 months at -20 ± 5 °C followed by 3 months at 5 ± 3 °C.	6 months	Complete
			Thermal Cycling 4	1 cycle of 1 month at -90 to -60 °C and 1 month at -50 ± 5 °C. 2 cycles of 1 month at -20 ± 5 °C and 1 month at -90 to -60 °C.	6 months	Complete
			Thermal Cycling 5	5 cycles of 4 days at -20 ± 5 °C and 1 day at 25 ± 2 °C/60 ± 5% RH. After cycling, move to -50 ± 5 °C until 2 months then transfer to -90 to -60 °C.	12 months	On-going
FC8273	June 2021	Process Performance Qualification, Puurs FC1 2.25 mL Fill, 30 µg dose. Supportive Stability	Long Term	-90 to -60 °C	12 months	On-going
			Additional	-20 ± 5 °C	3 months	Complete
			Additional	5 ± 3 °C	12 months	Complete
	Jan 22		Thermal Cycling 6	8 months at -90 to -60 °C and 4 months at 5±3 °C	4 months	Complete
FE4394	July 2021	Process Performance Qualification, Puurs FC2	Long Term	-90 to -60 °C	12 months	On-going
			Additional	-20 ± 5 °C	1 month	Complete
			Additional	5 ± 3 °C	12 months	Complete

Table 3.2.P.8.1-1. Summary of On-going Stability Studies

Lot Number	Stability Study Start	Drug Product Batch Use	Study Type	Storage Condition	Data Available	Study Status
	Jan 22	2.25 mL Fill, 30 µg dose. Supportive Stability	Thermal Cycling 6	7 months at -90 to -60 °C and 4 months at 5±3 °C	4 months	Complete
FJ5683	Sep 2021	Process Performance Qualification, Puurs FC1 2.25 mL Fill, 30 µg dose. Supportive Stability	Long Term	-90 to -60 °C	6 months	On-going
			Additional	-20 ± 5 °C	1 month	Complete
			Additional	5 ± 3 °C	6 months	Complete
FK5127	Sep 2021	Process Performance Qualification, Puurs WSL10 1.3 mL fill, 10 µg dose. Supportive Stability	Long Term	-90 to -60 °C	6 months	On-going
			Additional	-20 ± 5 °C	1 month	Complete
			Additional	5 ± 3 °C	6 months	Complete
FK5128	Sep 2021	Process Performance Qualification, Puurs WSL10 0.4 mL fill, 3 µg dose. Supportive Stability	Long Term	-90 to -60 °C	6 months	On-going
			Additional	-20 ± 5 °C	1 month	Complete
			Additional	5 ± 3 °C	6 months	Complete
FK5618	Oct 2021	Process Performance Qualification, Puurs WSL10 1.3 mL fill, 10 µg dose. Supportive Stability	Long Term	-90 to -60 °C	6 months	On-going
			Additional	-20 ± 5 °C	1 month	Complete
			Additional	5 ± 3 °C	6 months	Complete
FK5132	Oct 2021	Process Performance	Long Term	-90 to -60 °C	6 months	On-going
			Additional	-20 ± 5 °C	1 month	Complete

Table 3.2.P.8.1-1. Summary of On-going Stability Studies

Lot Number	Stability Study Start	Drug Product Batch Use	Study Type	Storage Condition	Data Available	Study Status
		Qualification, Puurs WSL9 2.25 mL fill, 30 µg dose. Supportive Stability	Additional	5 ± 3 °C	6 months	Complete
FM0703	Nov 2021	Process Performance Qualification, Puurs WSL9 1.3 mL fill, 10 µg dose. Supportive Stability	Long Term	-90 to -60 °C	6 months	On-going
			Additional	-20 ± 5 °C	1 month	Complete
			Additional	5 ± 3 °C	6 months	Complete
FP8748	Jan 2022	Process Performance Qualification, Puurs WSL5 2.25 mL Fill, 30 µg dose. Supportive Stability	Long Term	-90 to -60 °C	6 months	On-going
			Additional	-20 ± 5 °C	1 month	Complete
			Additional	5 ± 3 °C	6 months	Complete
2F1001A	Jan 2022	Process Performance Qualification BNT Marburg / Sanofi 2.25 mL Fill, 30 µg dose.	Long Term	-90 to -60 °C	3 months	On-going
			Additional	-20 ± 5 °C	3 months	Complete
			Additional	5 ± 3 °C	3 months	On-going
2F1003A	Jan 2022	Process Performance Qualification BNT Marburg / Sanofi 2.25 mL Fill, 30 µg dose.	Long Term	-90 to -60 °C	3 months	On-going
			Additional	-20 ± 5 °C	3 months	Complete
			Additional	5 ± 3 °C	3 months	On-going
FR5013	Feb 2022	Process Performance Qualification Puurs VC2 2.25 mL Fill, 30 µg dose. Supportive Stability	Long Term	-90 to -60 °C	6 months	On-going
			Additional	-20 ± 5 °C	1 month	Complete
			Additional	5 ± 3 °C	6 months	Complete

Table 3.2.P.8.1-1. Summary of On-going Stability Studies

Lot Number	Stability Study Start	Drug Product Batch Use	Study Type	Storage Condition	Data Available	Study Status
FR7348	Feb 2022	Process Performance Qualification Puurs WSL10 2.25 mL Fill, 30 µg dose. Supportive Stability	Long Term	-90 to -60 °C	6 months	On-going
			Additional	-20 ± 5 °C	1 month	Complete
			Additional	5 ± 3 °C	6 months	Complete
FW1374	Feb 2022	Process Performance Qualification Puurs WSL7 2.25 mL Fill, 30 µg dose. Supportive Stability	Long Term	-90 to -60 °C	6 months	On-going
			Additional	-20 ± 5 °C	1 month	Complete
			Additional	5 ± 3 °C	6 months	Complete
2F1004A	Feb 2022	Process Performance Qualification BNT Marburg / Sanofi 2.25 mL Fill, 30 µg dose.	Long Term	-90 to -60 °C	3 months	On-going
			Additional	-20 ± 5 °C	3 months	Complete
			Additional	5 ± 3 °C	3 months	On-going
FY3701	Mar 2022	Process Performance Qualification Puurs – 220 g RNA 2.25 mL Fill, 30 µg dose. Supportive Stability	Long Term	-90 to -60 °C	6 months	On-going
			Additional	-20 ± 5 °C	1 month	Complete
			Additional	5 ± 3 °C	3 months	Complete
GA5554	Apr 2022	Process Performance Qualification Puurs – 70 g RNA 2.25 mL Fill, 30 µg dose. Supportive Stability	Long Term	-90 to -60 °C	3 months	On-going
			Additional	-20 ± 5 °C	1 month	Complete
			Additional	5 ± 3 °C	3 months	Complete
FT9142	Feb 2022	Process Performance	Long Term	-90 to -60 °C	6 months	On-going
			Additional	-20 ± 5 °C	1 month	Complete

Table 3.2.P.8.1-1. Summary of On-going Stability Studies

Lot Number	Stability Study Start	Drug Product Batch Use	Study Type	Storage Condition	Data Available	Study Status
		Qualification – 0.4 mL Fill, 3 µg dose. Supportive Stability	Additional	5 ± 3 °C	3 months	Complete
FW9711	Mar 2022	Process Performance Qualification – 0.4 mL Fill, 3 µg dose. Supportive Stability	Long Term	-90 to -60 °C	6 months	On-going
			Additional	-20 ± 5 °C	1 month	Completed
			Additional	5 ± 3 °C	3 months	Complete
22AB004	Feb 2022	Process Performance Qualification Allergopharm / mibe 2.25 mL Fill, 30 µg dose.	Long Term	-90 to -60 °C	3 months	On-going
			Additional	-20 ± 5 °C	3 months	Complete
			Additional	5 ± 3 °C	3 months	On-going
22AC005	Mar 2022	Process Performance Qualification Allergopharm / mibe 2.25 mL Fill, 30 µg dose.	Long Term	-90 to -60 °C	3 months	On-going
			Additional	-20 ± 5 °C	3 months	Complete
			Additional	5 ± 3 °C	3 months	On-going
22AC006	Mar 2022	Process Performance Qualification Allergopharm / mibe 2.25 mL Fill, 30 µg dose.	Long Term	-90 to -60 °C	3 months	On-going
			Additional	-20 ± 5 °C	3 months	Complete
			Additional	5 ± 3 °C	3 months	On-going
GA5861	May 2022	Process Performance Qualification Puurs - 2.25 mL Fill, 30 µg dose. Supportive Stability	Long Term	-90 to -60 °C	3 months	On-going
			Additional	-20 ± 5 °C	1 month	Complete
			Additional	5 ± 3 °C	3 months	Complete

Table 3.2.P.8.1-1. Summary of On-going Stability Studies

Lot Number	Stability Study Start	Drug Product Batch Use	Study Type	Storage Condition	Data Available	Study Status

Abbreviations: RH = Relative Humidity; FC1 = Focus Cell 1 filling line; FC2 = Focus Cell 2 filling line; WSL9 = Washing and Sterilizing Line 9; WSL10 = Washing and Sterilizing Line 10; WSL5 = Washing and Sterilizing Line 5; VC2 = Vaccine Cell 2 filling line; WSL7 = Washing and Sterilizing Line 7

3.2.P.8.1.4. Protocol for Testing at the Long-Term Storage Condition of -90 to -60°C

Vials from primary and supportive PPQ drug product lots were stored at the long-term storage condition of -90 to -60 °C. Testing was performed according to the protocol indicated in Table 3.2.P.8.1-2 for the primary stability lots and Table 3.2.P.8.1-3 and Table 3.2.P.8.1-4 for the supportive PPQ stability lots.

Table 3.2.P.8.1-2. Protocol for BNT162b2 Tris/Sucrose Primary Drug Product Stored at the Long-Term Storage Condition of -90 to -60 °C [Puurs]

Analytical Procedure	Test Interval (Months)
Appearance (Visible)	0, 1, 2, 3, 4, 5, 6, 9, 12, 18, 24
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 3, 6, 12, 18, 24
Container Closure Integrity Test	3, 6, 12, 24
Endotoxin	0, 3, 6, 12, 24
Sterility	0, 12, 24

LNP = Lipid Nanoparticle

Table 3.2.P.8.1-3. Protocol for BNT162b2 Tris/Sucrose Supportive PPQ Drug Product, Stored at the Long-Term Storage Condition of -90 to -60 °C [Puurs]

Analytical Procedure	Test Interval (Months)
Appearance (Visible)	0,1[a], 3[a], 4[b], 6, 12, 18, 24
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 4[b], 6, 12, 18, 24
Container Closure Integrity Test	0, 12, 24
Endotoxin	
Sterility	

a. Additional timepoints were added
b. Additional timepoint only performed on lots FC8273 and FE4394.
LNP = Lipid Nanoparticle

Table 3.2.P.8.1-4. Protocol for BNT162b2 Tris/Sucrose PPQ Drug Product, 2.25 mL fill, Stored at the Long-Term Storage Condition of -90 to -60 °C [BioNTech Marburg / Sanofi; Allergopharma / mibe]

Analytical Procedure	Test Interval
Appearance (Visible)	0, 2W, 1M, 3M, 6M, 9M, 12M, 18M, 24M
Appearance (Visible Particulates)	
Potentiometry (pH)	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Subvisible Particles	
Capillary Gel Electrophoresis (RNA Integrity)	
Cell-based Flow Cytometry (In vitro expression)	
Container Closure Integrity Test	0, 6M, 12M, 24M
Endotoxin	
Sterility	

W = week, M = months, LNP = Lipid Nanoparticle

3.2.P.8.1.5. Protocols for Testing at the Additional Storage Conditions of -50 ± 5 °C, -20 ± 5 °C and 5 ± 3 °C

Vials from the primary drug product lots were stored at the additional storage condition of -50 ± 5 °C and tested per the protocol in Table 3.2.P.8.1-5. Subsequently it was determined that the -50 °C storage condition would not be required and this stability study was stopped at 1 month.

Vials from the primary and supportive PPQ drug product lots were stored at the additional storage conditions of -20 ± 5 °C per the protocols in Table 3.2.P.8.1-6. and Table 3.2.P.8.1-7. It was determined that the -20 °C storage condition is not suitable for long term storage therefore, stability protocols for primary stability were stopped at 6 months and supportive PPQ studies were stopped at either 1 or 3 months.

Vials for the primary and supportive PPQ drug product lots were stored at the additional storage condition of 5 ± 3 °C and tested per the protocols in Table 3.2.P.8.1-9 and Table 3.2.P.8.1-10.

Table 3.2.P.8.1-5. Protocol for BNT162b2 Tris/Sucrose Drug Product Stored at -50 ± 5 °C

Analytical Procedure	Test Interval (Months)
Appearance (Visible)	0, 1
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	

LNP = Lipid Nanoparticle

Table 3.2.P.8.1-6. Protocol for BNT162b2 Tris/Sucrose Primary Drug Product Stored at -20 ± 5 °C [Puurs]

Analytical Procedure	Test Interval (Months)
Appearance (Visible)	0, 1, 2, 3, 4, 5, 6
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 3, 6,
Container Closure Integrity Test	3, 6,
Endotoxin	0, 3, 6,

LNP = Lipid Nanoparticle

Table 3.2.P.8.1-7. Protocol for BNT162b2 Tris/Sucrose Supportive PPQ Drug Product Stored at -20 ± 5 °C [Puurs] [b]

Analytical Procedure	Test Interval (Months)
Appearance (Visible)	0, 1, 3[a]
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	

a. Three month time point only performed on lot FC8273.
b. The storage condition at -20 ± 5 °C has been terminated.

LNP = Lipid Nanoparticle

Table 3.2.P.8.1-8. Protocol for BNT162b2 Tris/Sucrose PPQ Drug Product, 2.25 mL fill, Stored at -20 ± 5 °C [BioNTech Marburg / Sanofi; Allergopharma / mibe][a]

Analytical Procedure	Test Interval
Appearance (Visible)	0, 2W, 1M, 3M
Appearance (Visible Particulates)	
Potentiometry (pH)	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Subvisible Particles	
Capillary Gel Electrophoresis (RNA Integrity)	
Cell-based Flow Cytometry (In vitro expression)	
Container Closure Integrity Test	0, 3M
Endotoxin	
Sterility	

a. The storage condition at -20 ± 5 °C has been terminated.

W = week, M = month, LNP = Lipid Nanoparticle

Table 3.2.P.8.1-9 Protocol for BNT162b2 Tris/Sucrose Primary Drug Product Stored at 5 ± 3 °C

Analytical Procedure	Test Interval
Appearance (Visible)	0, 2W, 1M, 6W, 2M, 3M, 4M, 5M, 6M
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 6M

W = Week, M = Month, LNP = Lipid Nanoparticle

Table 3.2.P.8.1-10. Protocol for BNT162b2 Tris/Sucrose Supportive PPQ Drug Product Stored at 5 ± 3 °C [Puurs]

Analytical Procedure	Test Interval (Months)
Appearance (Visible)	0, 1, 3, 6[a], 12[b]
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 3[c], 6[a], 12[b]
Container Closure Integrity	0, 3[c], 12[b]
Endotoxin	
Sterility	

LNP = Lipid Nanoparticle

a. Additional time point only performed on lots FJ5683, FK5127, FK5128, FK5618, FK5132, FM0703, FP8748, FR5013, FR7348 and FW1374.

b. Additional time point only performed on lots FC8273 and FE4394

c. The storage condition at 5 ± 3 °C has been shortened to end at 3 months.

Table 3.2.P.8.1-11. Protocol for BNT162b2 Tris/Sucrose PPQ Drug Product, 2.25 mL fill, Stored at 5 ± 3 °C [BioNTech Marburg / Sanofi; Allergopharma / mibe][a]

Analytical Procedure	Test Interval
Appearance (Visible)	0, 2W, 1M, 3M[a]
Appearance (Visible Particulates)	
Potentiometry (pH)	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Subvisible Particles	
Capillary Gel Electrophoresis (RNA Integrity)	
Cell-based Flow Cytometry (In vitro expression)	
Container Closure Integrity Test	0, 3M[a]
Endotoxin	
Sterility	

a. The storage condition at 5 ± 3 °C has been shortened to end at 3 months.

W = week, M = month, LNP = Lipid Nanoparticle

3.2.P.8.1.6. Protocol for Testing at the Thermal Stress Conditions of 25 ± 2°C/ 60 ± 5% RH and 30 ± 2°C/60 ± 5% RH

To study the effects of thermal stress conditions, two primary drug product lots, EX0490 and EW4564, were stored at 25 ± 2 °C/60 ± 5% RH and 30 ± 2 °C/65% ± 5 RH and tested per the protocols indicated in Table 3.2.P.8.1-12 and Table 3.2.P.8.1-13.

Table 3.2.P.8.1-12. Protocol for BNT162b2 Tris/Sucrose Drug Product at the Thermal Stress Condition of 25 ± 2°C/60 ± 5% RH

Analytical Procedure	Test Interval
Appearance (Visible)	0, 3D, 1W, 2W, 1M
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	

D = Day, W = Week, M = Month, LNP = Lipid Nanoparticle

Table 3.2.P.8.1-13. Protocol for BNT162b2 Tris/Sucrose Drug Product at the Thermal Stress Condition of 30 ± 2°C/65 ± 5% RH

Analytical Procedure	Test Interval
Appearance (Visible)	0, 3D, 1W, 2W, 1M
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	

D = Day, W = Week, M = Month, LNP = Lipid Nanoparticle

3.2.P.8.1.7. Protocol for Thermal Cycling Studies

To study the effects of temporary temperature excursions above the recommended storage temperature and the additional storage temperatures, thermal cycling studies were performed on three primary drug product lots according to the protocols indicated in Table 3.2.P.8.1-14 through Table 3.2.P.8.1-20. Subsequently it was determined that the -20 °C storage condition is not suitable for long term storage, therefore the thermal cycling 2, 3 and 4 studies were terminated after the 6 months time point.

To support storage of drug product for 3 months at 5 ± 3 °C (including up to 10 weeks at Point of Use), another thermal cycling study is conducted. In this study, three drug product lots are stored at -90 to -60 °C for up to 10 months, followed by storage at 2 – 8 °C for 4 months as indicated in Table 3.2.P.8.1-19

Table 3.2.P.8.1-14. Protocol for BNT162b2 Tris/Sucrose Drug Product Thermal Cycling 1: -20 ± 5 °C for 1 month then 2-8 °C for 6 months

Thermal Cycling Conditions: - Day 0, all inventory placed at -20 ± 5°C for 1 month. - At 1 month, samples pulled for testing and all other inventory transferred to 5 ± 3 °C for 6 months.	
Analytical Procedure	**Test Interval (Months)**
Appearance (Visible)	0, 1, 3, 4, 7
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 4, 7
Container Closure Integrity Test	4
Endotoxin	0, 4

LNP = Lipid Nanoparticle

Table 3.2.P.8.1-15. Protocol for BNT162b2 Tris/Sucrose Drug Product Thermal Cycling 2: -20 ± 5 °C for 2 months then 2-8 °C for 3 months

Thermal Cycling Conditions: - Day 0, all inventory placed at -20 ± 5°C for 2 months. - At 2 months, samples pulled for testing and all other inventory transferred to 5 ± 3 °C for 3 months.	
Analytical Procedure	**Test Interval (Months)**
Appearance (Visible)	0, 1, 2, 4, 5
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 5
Container Closure Integrity Test	5
Endotoxin	0, 5

LNP = Lipid Nanoparticle

Table 3.2.P.8.1-16. Protocol for BNT162b2 Tris/Sucrose Drug Product Thermal Cycling 3: -20 ± 5 °C for 3 months then 2-8 °C for 3 months

Thermal Cycling Conditions: - Day 0, all inventory placed at -20 ± 5°C for 3 months. - At 3 months, samples pulled for testing and all other inventory transferred to 5 ± 3 °C for 3 months.	
Analytical Procedure	**Test Interval (Months)**
Appearance (Visible)	0, 1, 2, 3, 5, 6
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 3, 6
Container Closure Integrity Test	3, 6
Endotoxin	0, 3, 6

LNP = Lipid Nanoparticle

Table 3.2.P.8.1-17. Protocol for BNT162b2 Tris/Sucrose Drug Product Thermal Cycling 4: -90 °C to -60 °C for 1 month then -50 ± 5 °C for 1 month, then 2 cycles of 1 month each at -20 ± 5 °C and -90 °C to -60 °C

Thermal Cycling Conditions: - Day 0, all inventory placed at -90 to -60 °C for 1 month. - At 1 month, all inventory transferred to -50 ± 5 °C for 1 month. - At 2 month, samples pulled for testing and all other inventory transferred to -20 ± 5 °C for 1 month. - At 3 month, all inventory transferred to -90 to -60 °C for 1 month. - At 4 month, samples pulled for testing and all other inventory transferred to -20 ± 5 °C for 1 month. - At 5 month, all inventory transferred to -90 to -60 °C for 1 month. - At 6 month, samples pulled for testing.	
Analytical Procedure	**Test Interval (Months)**
Appearance (Visible)	0, 2 , 4, 6
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 6

LNP = Lipid Nanoparticle

Table 3.2.P.8.1-18. Protocol for BNT162b2 Tris/Sucrose Drug Product Thermal Cycling 5: 5 cycles at -20 ± 5 °C for 4 days and 25 ± 2 °C/ 60 ± 5% RH for 1 day, then -50 ± 5 °C until 2 months and -90 to -60 °C

Thermal Cycling Conditions: - 5 cycles each consisting of 4 days at -20 ± 5 °C move to 25 ± 2 °C/60 ± 5% RH for 1 day (25 days total). - Samples pulled for testing after cycle 5 completed. All other inventory moved to -50 ± 5 °C until 2 months. - At 2 months, all inventory moved to -90 to -60 °C for the duration of the study and pulled for testing at 6, 12 and 24 months.	
Analytical Procedure	**Test Interval**
Appearance (Visible)	0, Cycle 5, 6M, 12M, 24M
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 6, 12, 24
Container Closure Integrity Test	12, 24

M = Months, LNP = Lipid Nanoparticle

Table 3.2.P.8.1-19. Protocol for BNT162b2 Tris/Sucrose Drug Product Thermal Cycling 6: -90 to -60 °C for up to 10 months[a], then 2-8 °C for 4 months

Thermal Cycling Conditions:
- Day 0, samples were pulled for T0 testing. Prior to Day 0, samples were stored at -90 to -60 °C for up to 10 months[a].
- All other inventory moved to 5±3 °C for the duration of the study.

Analytical Procedure	Test Interval (Months)
Appearance (Visible)	0, 1, 3, 4
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 3, 4
Container Closure Integrity Test	0, 4
Endotoxin	0, 4
Sterility	

a. The study is conducted on 3 drug product lots (EX0490, FC8273 and FE4394), which have been held at -90 to -60 °C for the following periods of time (as of the date of manufacture): 10 months (lot EX0490), 8 months (lot FC8273) and 7 months (lot FE4394).

LNP = Lipid Nanoparticle

3.2.P.8.1.8. Protocol for Photostability

To evaluate photostability, one primary drug product lot was exposed to ICH Q1B (option two) light conditions of 1.2 million lux hours of light and 200 watt h/m^2 of near ultraviolet (UV). Vials were orientated inverted for maximum light exposure in a light chamber set to 5 ± 3 °C, as it is not feasible to maintain the samples at the intended storage condition of -90 to -60 °C for this study and the 5 ± 3 °C condition is considered worst case exposure condition. Testing was performed according to the protocol shown in Table 3.2.P.8.1-20.

Table 3.2.P.8.1-20. Protocol for BNT162b2 Tris/Sucrose Photostability in Drug Product Vials

Analytical Procedure	Test Points
Appearance (Visible)	With Light Protection and Without Light Protection
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	

LNP = Lipid Nanoparticle

3.2.P.8.1.9. Summary of Stability Data

3.2.P.8.1.9.1. Summary of Stability Data at the Long-Term Storage Condition of -90 to -60 °C

The results obtained for BNT162b2 Tris/Sucrose drug product lots stored at the long-term storage condition of -90 to -60 °C are provided in Section 3.2.P.8.3 Long-Term Storage – Tris-Sucrose.

Summary of Results for Lots Manufactured at Puurs

- Eighteen months data are currently available for the three primary drug product lots manufactured by Pfizer, Puurs. All results met acceptance criteria in place at time of testing through the 18 month time point. At the 12 month time point, results for lot EW4564 and EW4565 do not meet the current acceptance criterion for RNA content, however this testing was performed using a previous version of the analytical method and results were within the acceptance criterion at the time of testing; therefore, no investigation was performed. As the 18 month time point results meet all of the current acceptance criteria, there is no impact to the stability of the drug product at the intended storage condition.

- Twelve months of stability data are available for PPQ lots FC8273, FE4394 and six months stability data is available for Puurs PPQ lot FJ5683, filled on lines FC1 and FC2 at 2.25 mL. All results available met acceptance criteria.

- Six months stability data are available for Puurs PPQ lots FK5127 and FK5618 filled at 1.3 mL and for lot FK5128, filled at 0.4 mL. These 3 lots were filled on the WSL10 line and all results met acceptance criteria.

- Six months stability data are available for Puurs PPQ lots FK5132 filled at 2.25 mL and FM0703 filled at 1.3 mL, for filling at Puurs on WSL9.

- For lots FP8748, FR5013, FR7348 and FW1374 filled at 2.25 ml on WSL5, VC2, WSL10 and WSL7 respectively, 6 months stability data are available Three month data is available for lot GA5554 and six month data is available lot FY3701, supporting the 70 - 220 g RNA lot size range for Tris/Sucrose filled at 2.25 mL.

- Stability results up to six months are available for lots FT9142 and FW9711 filled at 0.4 mL on WSL10 and WSL9, respectively.

For batch GA5861, the results of testing at 3 months time point are available. All results met the acceptance criteria, supporting the use of drug product intermediate with RNA concentration of 0.5 mg/mL during manufacture of the final drug product.

Summary of Results for Lots Manufactured at BioNTech Marburg/Sanofi

All results of the 2-week, 1- and 3-month time points of the BioNTech Marburg/Sanofi PPQ drug product lots 2F1001A, 2F1003A and 2F1004A stored at the long-term storage condition met the acceptance criteria.

Summary of Results for Lots Manufactured at Allegopharma/mibe

All results of the 2-week, 1- and 3-month time points of the Allergopharma/mibe PPQ drug product lots 22AB004, 22AC005, and 22AC006 stored at the long-term storage condition met the acceptance criteria.

Overall, the data indicate that there have been no significant changes in terms of quality, purity, or strength for the drug product.

3.2.P.8.1.9.2. Summary of Stability Data at the Additional Storage Conditions of -50 ± 5 °C, -20 ± 5 °C and 5 ± 3 °C

-50 ± 5 °C Stability

The results obtained for BNT162b2 Tris/Sucrose primary drug product lots stored at -50 ± 5 °C are provided in Section 3.2.P.8.3 Additional Storage Conditions – Tris-Sucrose. The stability data at study completion at 1 month storage met acceptance criteria.

-20 ± 5 °C Stability

The results obtained for BNT162b2 Tris/Sucrose drug product lots stored at -20 ± 5 °C are provided in Section 3.2.P.8.3 Additional Storage Conditions– Tris-Sucrose.

Summary of Results for Lots Manufactured at Puurs

- For the three primary drug product lots manufactured at Puurs, all results met acceptance criteria through study completion at 6 months, except for LNP size for lot EW4564. Increasing LNP size was observed for all 3 lots. These results together with those for thermal cycling studies 1, 2 and 3, summarized in Section 3.2.P.8.1.9.4, demonstrate that stability at -20 ± 5 °C is limited.

- For Puurs PPQ lots FC8273, FE4394 and FJ5683 filled on filling lines FC1 and FC2 at 2.25 mL fill volume, stability data at three months, 1 month and 1 month, respectively, met acceptance criteria. These studies are complete as they were terminated early as previous results demonstrated limited stability at -20 ± 5 °C.

- For Puurs PPQ lots FK5127, FK5128 and FK5618, filled on filling line WSL10 at 1.3 ml, 0.4 mL and 1.3 mL, respectively, stability data at 1 month met acceptance criteria. These studies are complete as they were terminated early as previous results demonstrated limited stability at -20 ± 5 °C.

- Stability data at 1 month met acceptance criteria for Puurs PPQ lots FK5132 filled at 2.25 mL and FM0703 filled at 1.3 mL, for filling at Puurs on WSL9. These studies are complete as they were terminated early as previous results demonstrated limited stability at -20 ± 5 °C.

- For Puurs PPQ lots FP8748, FR5013, FR7348 and FW1374 filled at 2.25 mL on filling lines WSL5, VC2, WSL10 and WSL7 respectively, one month stability data at -20°C is available. These studies are complete as they were terminated early as previous results demonstrated limited stability at -20 ± 5 °C.

- One month results are available for lot GA5554 and lot FY3701, supporting the70 - 220 g RNA lot size range for Tris/Sucrose filled at 2.25 mL. These studies are complete as they were terminated early as previous results demonstrated limited stability at -20 ± 5 °C.

- For PPQ lot FT9142 and lot FW9711, filled at 0.4 mL on WSL10 and WSL9 respectively 1 month stability data is available and met acceptance criteria.

- For batch GA5861, the results of testing at 1 month time point are available. All results meet the acceptance criteria, supporting the use of drug product intermediate with RNA concentration of 0.5 mg/mL during manufacture of the final drug product.

Summary of Results for Lots Manufactured at Marburg/Sanofi

All results of the 2-weeks, 1-month and 3-month time points of the BioNTech Marburg/Sanofi PPQ drug product lots 2F1001A, 2F1003A and 2F1004A stored at -20 ± 5 °C met the acceptance criteria.

Summary of Results for Lots Manfuactured at Allergopharma/mibe

All results of the 2-weeks, 1-month and 3 months time points of the Allergopharma/mibe PPQ drug product lots 22AB004, 22AC005, and 22AC006 stored at -20 ± 5 °C met the acceptance criteria.

5 ± 3 °C Stability

The results obtained for BNT162b2 Tris/Sucrose drug product lots stored at 5 ± 3 °C are provided in Section 3.2.P.8.3 Additional Storage Conditions – Tris-Sucrose.

Summary of Results for Lots Manufactured at Puurs

- For the three primary drug product lots manufactured at Puurs, all data met acceptance criteria through four months storage. Increasing LNP size and polydispersity were observed with OOS results for polydispersity for lots EX0490 and EW4564 at 5 months and for all 3 lots (EX0490, EW4564 and EW4565) at 6 months. Additionally, decreasing In Vitro Expression (IVE) levels were observed with OOS results for lot EX0490 at 6 months.

- For Puurs PPQ lots FC8273 (filling line FC1), FE4394 (filling line FC2) and FJ5683 stability data at 6 months met acceptance criteria except for LNP size and polydispersity. Questionable results were obtained due to System Suitability Test (SST) failure for lot FC8273 at both 6 and 12 months. For batches FC8273 (filling line FC1), FE4394 (filling line FC2), an out of specification (OOS) for IVE was obtained at 12 months timepoint against the acceptance criteria of ≥ 43% Cells Positive. For batch FC8273, no reportable result was obtained for sterility at 12 months due to clogging of the filter during testing and an additional OOS was observed for RNA integrity. No retests were performed as the 6 and 12 months timepoints are not representative for the commercial label claim.

- For Puurs PPQ lots FK5127 and FK5618, filled on filling line WSL10 at 1.3 mL, and PPQ lot FK5128, filled on filling line WSL10 at 0.4 mL, stability data at 6 months met acceptance criteria, except for one OOS IVE result for batch FK5128 at the 6 months timepoint. For all three lots (FK5127, FK5618 and FK5128) no reportable results were obtained due to SST failure for Dynamic Light Scattering (DLS) as a result of too high %RSD (Residual Standard Deviation). No retests were performed as the 6-months timepoint is not representative for the commercial label claim.

- For Puurs PPQ lots FK5132 and FM0703, filled on filling line WSL9 at 2.25 mL and at 1.3 mL respectively , stability data at 3 months met acceptance criteria. At the 6 months timepoint, questionable results were obtained due to SST failure for DLS with both batches. No retests were performed as the 6 months timepoint is not

representative for the commercial label claim. For batch FM0703, an out of specification for IVE was obtained at 6 months timepoint against the acceptance criteria of ≥ 43% Cells Positive. As these timepoints are not representative for the commercial label claim, no re-test was performed.

- For Puurs PPQ lot FP8748, FR5013, FR7348 and FW1374 filled at 2.25 mL on WSL5, VC2, WSL10 and WSL7 respectively, stability data at 3 months met acceptance criteria. At the 6 months timepoint, questionable results were obtained due to SST failure for DLS with al four batches. No retests were performed as the 6 months timepoint is not representative for the commercial label claim. For batch FW1374 an out of specification for IVE was obtained at 6 months timepoint against the acceptance criteria of ≥ 43% Cells Positive. As these timepoints are not representative for the commercial label claim, no re-test was performed.

- Data at three months is available for lot GA5554 and FY3701, supporting the 70 - 220 g RNA lot size range for Tris/Sucrose filled at 2.25 mL.

- Up to 3 months stability results are available for lots FT9142 and FW9711 filled at 0.4 mL on WSL10 and WSL9, respectively. All results for batch FT9142 met the acceptance criteria. An out of specification was observed for IVE (42 %) for lot FW9711 at 3 months. Based on the investigation, a special cause related to the sample handling has been identified as the most probable root cause. The investigation indicated that the stability study in scope for lot FW9711 is trending atypically and is not representative of the drug product with a fill volume of 0.4 mL.

- Three months data is available for lot GA5554 and lot FY3701, supporting the 70 - 220 g RNA lot size range for Tris/Sucrose filled at 2.25 mL. Three months timepoint is available for lot GA5861, supporting the 0.5 mg/mL drug product intermediate.

Summary of Results for Lots Manufactured at BioNTech Marburg/Sanofi

Three months results are available for the BioNTech Marburg/Sanofi PPQ drug product lots 2F1001A, 2F1003A and 2F1004A stored at 5 ± 3 °C. After 3 months for lots 2F1001A and 2F1004A the results for IVE dropped below the acceptance criterion whereas lot 2F1003A was within the limit. An investigation was carried out, but as this timepoint is not representative for the commercial label claim, no re-test was performed. All other parameters tested up to the 3 months time point for these lots met the acceptance criteria.

Summary of Results for Lots Manufactured at Allergopharma/mibe

All results of the 2-weeks, 1-month and 3 months time points of the Allergopharma/mibe PPQ drug product lots 22AB004, 22AC005, and 22AC006 stored at 5 ± 3 °C met the acceptance criteria.

These results demonstrate that stability of Tris/Sucrose drug product is limited to approximately 3 months storage at 5 ± 3 °C, supporting 2 weeks storage during internal manufacturing operations and 10 weeks storage at the point of use. These results also

demonstrate that these assays detect changes in BNT162b2 drug product indicative of stability.

3.2.P.8.1.9.3. Summary of Stability Data at the Thermal Stress Storage Conditions of 25 ± 2 °C/60 ± 5% RH and 30±2 °C/65 ± 5% RH

To support short term temperature excursions, BNT162b2 Tris/Sucrose primary drug product lots were exposed to the thermal stress conditions of 25 ± 2 °C/60 ± 5% RH and 30 ± 2 °C/ 65 ± 5% RH. The results obtained are provided in Section 3.2.P.8.3 Thermal Stress and Cycling – Tris-Sucrose.

At 25 ± 2 °C/60 ± 5% RH storage, the stability data at 2 weeks storage met acceptance criteria. At 1 month storage, LNP polydispersity for lot EW4564 and RNA integrity for lots EX0490 and EW4564 did not met acceptance criteria. Additionally, the IVE level dropped precipitously after 2 weeks to 1 month storage and RNA integrity showed a trend towards lower values.

At 30 ± 2 °C/65 ± 5% RH storage, the stability data at 3 days storage met acceptance criteria. At 1 week storage, IVE expression for lot EX0490 did not meet the acceptance criterion. At 2 weeks storage, RNA integrity for lot EW4564 did not meet the acceptance criterion. At 1 month storage, IVE expression for lot EX0490 and RNA integrity for lots EX0490 and EW4564 did not meet acceptance criteria.

The changes observed in the IVE and RNA integrity results with storage at 25 ± 2 °C/60 ± 5% RH and 30 ± 2 °C/60 ± 5% RH indicate that BNT162b2 Tris/Sucrose drug product has limited stability at these thermal stress conditions.

3.2.P.8.1.9.4. Summary of Stability Data at the Thermal Cycling Storage Conditions

A total of 6 thermal cycling studies are being performed.

Thermal Cycling 1, 2 and 3

The first three cycling studies are evaluating storage at -20 ± 5 °C for 1 month, 2 months and 3 months, respectively, followed by storage at 2-8 °C for the remainder of the study. This study was set up to support long-term storage and transport at -20 °C followed by short term storage at 2-8 °C, to facilitate handling and storage at point of use. These studies used the same samples as those for the additional storage condition at -20 ± 5°C storage for up to 3 months, as detailed in Section 3.2.P.8.1.5, prior to storage at 2–8 °C.

All three thermal cycling studies consistently showed increasing LNP size and polydispersity and decreasing RNA encapsulation, RNA integrity and IVE levels, including not meeting the acceptance criteria at various timepoints for LNP size and polydispersity and IVE levels. These results demonstrate that stability is limited to a combined 1 month storage at -20 °C followed by 2 months storage at 2-8 °C.

Based on these results, storage and transport is predominantly restricted to temperatures of -90 to -60 °C, with alternative shipping conditions restricted to ≤ 48 hours at -20 °C and ≤ 80 hours at 2-8 °C, as detailed in Section 3.2.P.3.3 Fill Finish – Tris-Sucrose – Puurs.

Thermal Cycling 4

The results obtained for the BNT Tris/Sucrose primary drug product lots cycled through 1 cycle of -90 to -60 °C for 1 month, then transfer to -50 ± 5 °C for 1 month and 2 cycles of -20 ± 5 °C / -90 to -60 °C for one month each, for a total of six months, are provided in Section 3.2.P.8.3 Thermal Stress and Cycling – Tris-Sucrose. All results met acceptance criteria through study completion.

Thermal Cycling 5

The results obtained for the BNT Tris/Sucrose primary stability lots cycled through 5 cycles of 4 days at -20 ± 5 °C and 1 day at 25 ± 2 °C/60 ± 5% RH followed by storage at -50 ± 5 °C until 2 months and then transferred to -90 to -60 °C for the remainder of the study are provided in Section 3.2.P.8.3 Thermal Stress and Cycling – Tris-Sucrose. All results met acceptance criteria through the five cycles, followed by storage at -50 ± 5 °C until 2 months, followed be storage at -90 to -60 °C for 10 months.

Thermal Cycling 6

The results obtained for three lots of drug product stored for up to 10 months at –90 to –60 °C then placed at 2-8 °C for testing at 0, 1, 3 and 4 months are provided in Section 3.2.P.8.3 Thermal Stress and Cycling – Tris-Sucrose. All results met the acceptance criteria and provide additional support for commercial handling conditions of long-term storage at -90 to -60 °C followed by 10 weeks at 2-8 °C.

3.2.P.8.1.9.5. Summary of Photostability in Drug Product Vials

The results obtained for the BNT Tris/Sucrose primary stability lot EW4564 exposed to ICH Q1B (option two) light conditions are provided in Section 3.2.P.8.3 Stability Data – Photostability – Tris-Sucrose. Results for drug product not protected from light were similar to results for drug product protected from light. A slight decrease in In Vitro Expression and RNA integrity were observed for samples exposed to light as compared to those protected from light, however this is not unexpected, and all results generated met acceptance criteria. The data indicates that drug product does not need to be protected from light.

3.2.P.8.1.10. Shelf Life and Conclusions

The stability data obtained to date for the BNT162b2 Tris/Sucrose drug product support 18 months expiry dating when stored at the recommended long-term storage condition of -90 to -60 °C. This shelf life is based on 18 months stability data for the three BNT162b2 Tris/Sucrose primary drug product lots, up to 12 months stability data from PPQ lots manufactured at Puurs and 24 weeks Tris/Sucrose development stability data. Additionally, the stability data generated to date support short term storage at 5 ± 3 °C for up to 3 months, within the 18-month shelf life, supporting the additional storage conditions of 2-8 °C for 10 weeks at the point of use.

REVIEWED
By s22 at 3:31 pm, Nov 07, 2022

Rec 07NOV2022
2211003541 Prot
2211003542 Initial

Document 19

sciensano ← LIMS

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BELAC 031-TEST

Quality of Vaccines and Blood Products

NATIONAL • CONTROL • LABORATORY sciensano BELGIUM

EU OFFICIAL CONTROL AUTHORITY BATCH RELEASE CERTIFICATE FOR IMMUNOLOGICAL PRODUCTS

EU/EEA OFFICIAL CONTROL AUTHORITY BATCH RELEASE CERTIFICATE – Finished Product

Examined under Article 114 of Directive 2001/83/EC as amended by Directive 2004/27/EC (Immunological Medicinal Products) and in accordance with the Administrative Procedure for Official Control Authority Batch Release.

Trade name	**COMIRNATY Original/Omicron BA.1** ✓
International Non-proprietary Name / Ph.Eur. name / Common name	**Pandemic COVID-19 Vaccine (mRNA)/ Bivalent 15µg/15µg (Original and Omicron BA.1)**
Batch numbers appearing on the package and other identification numbers associated with this batch[1]	**GE1643** ✓
Type of container	**Vial**
Total number of containers in this batch	s47
Number of doses per container	**6 doses**
Date of start of period of validity	**25 May 2022**
Date of expiry	**30 April 2023** ✓
Marketing Authorisation number (member state / EU) issued by	**EU/1/20/1528/006-007**
Name and address of manufacturer	**Pfizer Manufacturing Belgium NV / 2870 Puurs, Belgium**
Name and address of marketing authorisation holder if different	**BioNTech Manufacturing GmbH An der Goldgrube 12 55131 Mainz, Germany**

This batch has been examined using documented procedures which form part of a quality system which is in accordance with the ISO/IEC 17025 standard.
This examination is based on the relevant EU OCABR guideline for this product.

This batch is in compliance with the approved specifications laid down in the relevant European Pharmacopoeia monographs and the above marketing authorisation and is released.

Signature **Date of issue**	s22 Digitally signed by s22 Date: 2022.09.02 15:42:06 +02'00' ← LIMS
Name and function of signatory	**p.o.** s22 **Quality of Vaccines and Blood Products Service**

Certificate number: BE/22/1997 ← LIMS

[1] Such as batch number of final bulk

Sciensano Rue Juliette Wytsmanstraat 14 1050 Brussels Belgium
T + 32 2 642 51 11 F + 32 2 642 50 01 info@sciensano.be www.sciensano.be

REVIEWED
By s22 at 2:58 pm, Nov 08, 2022

Pfizer Manufacturing Belgium NV
Rijksweg 12
2870 Puurs
Belgium

Date: 23-Aug-2022

TGA Protocol Checklist used: QPulse Bio-BRU-Form-65 Version 8 8.11.22 s22

Validator - Please note anomalies (incorrect spec & missing DS tests) as bookmarked. 8.11.22 s22

Sciensano
Quality of Vaccines and Blood Products
Juliette Wytsmanstraat 14
B1050 Brussels
Belgium

Protocol passed in LIMS (as per s22 Company to be sent a single email for all protocols currently on hand (8.11.22) outlining the anomalies which are consistent across all protocols. 8.11.22 s22

Email sent 11.11.22 D22-6083541 s22

Please consider this as an official request for Official Control Authority Batch Release (OCABR) and WHO release for the following product:

Follow up email sent D22-6236511 19.12.22 s22

No response received. However, enquiry has now been CLOSED in agreeance with s22 - Sponsor notified by email - See D23-5484447 20.6.23 s22

Trade Name:	COMIRNATY
Type of Container:	Vial
Storage Conditions:	-90°C to -60°C
Marketing Authorisation Holder:	BioNTech Manufacturing GmbH, An der Goldgrube 12, 55131 Mainz Germany
Batch Number:	GE1643 ✓
Presentation:	Bivalent [Original and Omicron BA.1] AUSTR 394890
Process Variation:	Adult (15/15 µg) ✓
Date of Filling:	08-Jun-2022
Expiry Date:	30-Apr-2023 ✓
Total Quantity:*	s47
Site of Manufacture:	Pfizer Manufacturing Belgium NV, PGS Puurs, Rijksweg 12, 2870 Puurs, Belgium

*No deviations were raised in context of yield for the batch. Therefore, there were no exceedance of the yield limits

Please find enclosed the lot release protocol and genealogy diagram for this batch. The lot release protocol has been reviewed by a Qualified Person and found to be satisfactory. The OMCL performing OCABR has been notified of all relevant approved variations that have an impact on product specifications or on data supplied in section 3 of this protocol as described in the EU administrative procedure for OCABR.

In addition, certification by qualified person that the above COMIRNATY batch was manufactured following all national requirements and complies with WHO good manufacturing practices for pharmaceutical products: main principles; WHO good manufacturing practices for biological products; and WHO Guidelines for independent lot release of vaccines by regulatory authorities.

- WHO Technical Report Series, No. 986, Annex 2.
- WHO Technical Report Series, No. 999, Annex 2.
- WHO Technical Report Series, No. 978, Annex 2

Prepared By:
s22 s22
02 Sep 2022 03:48:049-0400
REASON: I approve this document.
9633d8dc-763c-4d96-aa2e-f081e9d901ed
QP Delegate

Approved By:
s22 s22
02 Sep 2022 04:11:012-0400
REASON: I approve this document.
d007747d-0c63-4417-9d1e-20158a2a4d4b
QP Delegate

DocUUID : 60584476-420c-43ce-9d1e-5b03ae4fb9f0

REASON FOR SUBMISSION:
For Release

Lot Number: GE1643
Trade Name of Product: COMIRNATY
Licensed Name of Product: COMIRNATY
Marketing Authorisation Holder Name and Address: BioNTech Manufacturing GmbH, An der Goldgrube 12, 55131 Mainz, Germany
Manufacturing Site: Pfizer Manufacturing Belgium NV, PGS Puurs, Rijksweg 12, 2870 Puurs, Belgium
Marketing Authorisation Number: EU/1/20/1528/006

Date of Manufacture: 25-May-2022 LIMS **Date of Expiry:** 30-Apr-2023
Date of Fill: 08-Jun-2022

Product Information:

Drug Substance Target Concentration: s47 mg/mL

LOT GENEALOGY

Component Description	Batch Number	Date of Manuf.	Manufacture Site	Quantity
Working Cell Bank	32134855	01-Jun-2021	Sandoz GmbH / Novartis, Austria	N/A
Master Cell Bank Omicron	FT1817	24-Dec-2021	Pfizer Andover	N/A
DNA Plasmid linearized	B625536	08-Oct-2021	Sandoz GmbH / Novartis, Austria	s47
DNA Plasmid linearized Omicron	FT0614	06-Jan-2022	Pfizer Andover	s47
BNT162b2 Drug Substance	2236630_MB0084 / 1087247	31-Jan-2022	BioNTech Manufacturing Marburg GmbH / Rentschler Biopharma SE	s47
BNT162b2 Omicron Drug Substance	2241554_MB0010	06-Feb-2022	BioNTech Manufacturing Marburg GmbH	
BNT162b2 Omicron Drug Substance	2241554_MB0011	07-Feb-2022	BioNTech Manufacturing Marburg GmbH	
LNP Fabrication and Bulk Drug Product Formulation*	GE1848	25-May-2022	Pfizer Puurs	
Semi-Finished Goods	GE1674	25-May-2022	Pfizer Puurs	
Drug Product Packaging	GE1643	25-May-2022	Pfizer Puurs	

see below
DNA Linearized Plasmid (2x lots) Not mentioned in protocol other than in lot genealogy and genealogy flowchart 8.11.22 s22
Semi-Finished Goods - Not mentioned in protocol other than in lot genealogy and genealogy flowchart 8.11.22 s22

*No LNP bulk stored as drug product intermediate was used during manufacturing.

MCB Omicron FT1817
Approved in PM-2022-03551-1-2 Evaluation D22-5836308 Approval D22-6018548

Checklist update has been flagged & added to QPulse (CR522) 8.11.22 s22

Lot Number: GE1643

Licensed Name of Product: COMIRNATY

FILL INFORMATION

Container Type:	Vial	**Volume per container:**	2.25 mL
Approved Storage Period:	12 months	**Storage Temperature:**	-90°C to -60°C
Number of containers for release:	s47	**Number of Doses per container:**	6
Single human dose strength:	15/15 µg/Dose	**Start Date of period of Validity:**	Date of Manufacture

Certification by qualified person taking the overall responsibility for production and control of the product was manufactured and tested according to the procedures approved by the competent authorities and complies with the quality requirements. This includes that, for any materials derived from ruminants (bovine, ovine, caprine) used in the manufacture and/or formulation of the batch of product specified above, all measures have been taken to demonstrate compliance with Directive 2001/83/EC and amending Directives 2003/63/EC and 2004/27/EC.

In addition, the OMCL performing OCABR has been notified of all relevant approved variations that have an impact on product specifications or on data supplied in section 3 of this protocol as described in the EU administrative procedure for OCABR.

Prepared By:

s22 s22

02 Sep 2022 03:48:049-0400

REASON: I approve this document.

9633d8dc-763c-4d96-aa2e-f081e9d901ed

Approved By:

s22 s22

02 Sep 2022 04:11:012-0400

REASON: I approve this document.

d007747d-0c63-4417-9d1e-20158a2a4d4b

Lot Number:GE1643

Licensed Name of Product: COMIRNATY

Table 1. **Filled Vaccine Quality Control Tests**

Test	Test Method	Specification	Date of Test	Result
Appearance	Appearance (Visual)	White to off-white suspension	22-Jun-2022	MEETS TEST
Appearance (Visible Particulates)	Appearance (Particles)	May contain white to off-white opaque amorphous particles	22-Jun-2022	MEETS TEST
Subvisible Particles	Subvisible Particulate Matter	Particles >= 10µm: <= 6000 per container	20-Jun-2022	s47
Subvisible Particles	Subvisible Particulate Matter	Particles >= 25µm: <= 600 per container	20-Jun-2022	
pH	Potentiometry	6.9 - 7.9	22-Jun-2022	7.5
Osmolality	Osmometry	s47	20-Jun-2022	s47
LNP Size	Dynamic Light Scattering (DLS)		17-Jun-2022	
LNP Polydispersity	Dynamic Light Scattering (DLS)		17-Jun-2022	
RNA Encapsulation	Fluorescence assay		24-Jun-2022	
RNA content	Fluorescence assay		24-Jun-2022	
ALC-0315 content	HPLC-CAD		21-Jun-2022	
ALC-0159 content	HPLC-CAD		21-Jun-2022	
DSPC content	HPLC-CAD		21-Jun-2022	
Cholesterol content	HPLC-CAD		21-Jun-2022	
Container content for injections	Vial Content (Volume)		20-Jun-2022	
Lipid identities	HPLC-CAD	Retention times consistent with references (ALC-0315, ALC-0159, Cholesterol, DSPC)	21-Jun-2022	MEETS TEST

LIMS

s47

All results comply

Lot Number: GE1643

Licensed Name of Product: COMIRNATY

Table 1 (Continued) Filled Vaccine Quality Control Tests

Test	Test Method	Specification	Date of Test	Result
Identity of encoded RNA sequence	ddPCR	Identity confirmed: BNT162b2	11-Aug-2022	Identity Confirmed
Identity of encoded RNA sequence	ddPCR	Identity confirmed: BNT162b2 Omicron	11-Aug-2022	Identity Confirmed
RNA Ratio	ddPCR	s47 %: BNT162b2	11-Aug-2022	s47 LIMS
RNA Ratio	ddPCR	s47 %: BNT162b2 Omicron	11-Aug-2022	
In Vitro Expression	Cell-based Flow Cytometry	s47	23-Jun-2022	
RNA Integrity	Capillary Gel Electrophoresis	s47	21-Jun-2022	
Bacterial Endotoxin	Endotoxin (LAL)	s47	18-Jun-2022	

Abbreviations: LNP = Lipid Nanoparticles; HPLC = High-Performance Liquid Chromatography; CAD = Charged Aerosol Detector; ddPCR = droplet digital Polymerase Chain Reaction; LAL = Limulus Amebocyte Lysate; EU = Endotoxin Unit

Filled Vaccine Quality Control Tests (cont.)

All results comply

Sterility

Method: Membrane Filtration

Type: Final Container

Container: Sterility-20 mL/medium (40 vials)

Date On Test	Medium/Temperature	Date Off Test	Specification	Test Result
13-Jun-2022	Thioglycollate 30°C-35°C	28-Jun-2022	No growth observed	No growth observed
	Soybean Casein Digest 20°C-25°C		No growth observed	No growth observed

Lot Number: GE1643

Licensed Name of Product: COMIRNATY

Table 2: Fill Vaccine In-Process Tests – Fill Weight Measurements

Pump	Minimum	Maximum	Mean
1	s47		
2			
3			
4			
5			
6			
7			
8			

Acceptance Criteria s47
All measurements were within limits.

Lot Number: GE1643
Licensed Name of Product: COMIRNATY

BNT162b2 LNP Fabrication

LNP Lot Number: GE1848

Table 1: In-Process Tests (IPT-C)

Test	Test Method	Specification	Date of Test	Result
pH s47	Potentiometry	s47	03-Jun-2022	s47
pH s47	Potentiometry		04-Jun-2022	
RNA Content s47 s47	Fluorescence assay		06-Jul-2022	

Lot Number: GE1643

Licensed Name of Product: COMIRNATY

BNT162b2 Drug Substance

Lot Number: 2236630_MB0084

Date of Manufacture: 31-Jan-2022 ✓ **Date of Expiry:** 30-Jul-2022 ✓

Storage Temperature: - 25°C to - 15°C **Approved Storage Period:** 6 months

Consumed Quantity: s47

Table 1. Drug Substance In-Process Tests (IPT-C)

Test	Test Method	Specification ✓	Date of Test	Result ✓
RNA Content s47 s47	UV Spectroscopy	s47	31-Jan-2022	s47
RNA Content s47 s47	UV Spectroscopy		31-Jan-2022	

Table 2. Drug Substance Quality Control Tests

Test	Test Method	Specification ✓	Date of Test	Result ✓
Clarity	Appearance (Clarity)	<= 6 NTU	04-Feb-2022	0 NTU
Coloration	Appearance (Coloration)	Not more intensely colored than level 7 of the brown (B) color standard	04-Feb-2022	complies
pH	Potentiometry	7.0 +/- 0.5	04-Feb-2022	6.9
Content (RNA Concentration)	UV Spectroscopy	s47	16-Mar-2022	s47
Identity of Encoded RNA Sequence	RT-PCR	Identity confirmed	09-Feb-2022	Complies
RNA Integrity	Capillary Gel Electrophoresis	s47	17-Mar-2022	s47
5'- Cap	RP-HPLC		25-Mar-2022	
Poly(A) Tail	ddPCR		21-Mar-2022	
Residual DNA Template	qPCR		10-Mar-2022	
Residual dsRNA	Immunoblot		21-Mar-2022	
Bacterial Endotoxin	Endotoxin (LAL)		02-Feb-2022	
Bioburden	Bioburden		07-Feb-2022	

Test missing - see below

Abbreviations: NTU = Nephelometric Turbidity Units; RT-PCR = Reverse Transcription PCR; dd-PCR = droplet digital PCR; IP-RP-HPLC = Ion-Pair Reversed-Phase HPLC; qPCR = quantitative PCR; dsRNA = double stranded RNA; LAL = Limulus Amebocyte Lysate; EU = Endotoxin Unit; CFU = Colony Forming Unit

Test Missing
Poly(A) Tail Length (spec - Poly(A) Tail Length Confirmed)

Added to DS release tests for Bivalent formulation in PM-2022-03551-1-2 and for the Monovalent formulation in PM-2021-04026-1-2

8.11.22 s22

All results comply

Lot Number: GE1643

Licensed Name of Product: COMIRNATY

BNT162b2 Omicron Drug Substance

Lot Number: 2241554_MB0010

Date of Manufacture: 06-Feb-2022 ✓ **Date of Expiry:** 06-Aug-2022 ✓

Storage Temperature: - 25°C to - 15°C **Approved Storage Period:** 6 months

Consumed Quantity: s47

Table 1. Drug Substance In-Process Tests (IPT-C)

Test	Test Method	Specification ✓	Date of Test	Result ✓
RNA Content s47 s47	UV Spectroscopy	s47	05-Feb-2022	s47
RNA Content s47 s47	UV Spectroscopy		05-Feb-2022	

Table 2. Drug Substance Quality Control Tests

Test	Test Method	Specification ✓	Date of Test	Result ✓
Clarity	Appearance (Clarity)	<= 6 NTU	08-Feb-2022	<= 6 NTU
Coloration	Appearance (Coloration)	Not more intensely colored than level 7 of the brown (B) color standard	08-Feb-2022	complies
pH	Potentiometry	7.0 +/- 0.5	07-Feb-2022	7.0
Content (RNA Concentration)	UV Spectroscopy	s47	05-Feb-2022	s47
Identity of Encoded RNA Sequence	RT-PCR	Identity confirmed	15-Feb-2022	Complies
RNA Integrity	Capillary Gel Electrophoresis	s47	08-Feb-2022	s47
5'- Cap	RP-HPLC		10-Feb-2022	
Poly(A) Tail	ddPCR		15-Feb-2022	
Residual DNA Template	qPCR		09-Feb-2022	
Residual dsRNA	Immunoblot		14-Feb-2022	
Bacterial Endotoxin	Endotoxin (LAL)		08-Feb-2022	
Bioburden	Bioburden		08-Feb-2022	

Test missing - see below

Abbreviations: NTU = Nephelometric Turbidity Units; RT-PCR = Reverse Transcription PCR; dd-PCR = droplet digital PCR; IP-RP-HPLC = Ion-Pair Reversed-Phase HPLC; qPCR = quantitative PCR; dsRNA = double stranded RNA; LAL = Limulus Amebocyte Lysate; EU = Endotoxin Unit; CFU = Colony Forming Unit

Test Missing
Poly(A) Tail Length (spec - Poly(A) Tail Length Confirmed)

Added to DS release tests for Bivalent formulation in PM-2022-03551-1-2 and for the Monovalent formulation in PM-2021-04026-1-2

8.11.22 s22

All results comply

Lot Number: GE1643

Licensed Name of Product: COMIRNATY

BNT162b2 Omicron Drug Substance

Lot Number: 2241554_MB0011

Date of Manufacture: 07-Feb-2022 **Date of Expiry:** 07-Aug-2022

Storage Temperature: - 25°C to - 15°C **Approved Storage Period:** 6 months

Consumed Quantity: s47

Table 1. Drug Substance In-Process Tests (IPT-C)

Test	Test Method	Specification	Date of Test	Result
RNA Content s47 s47	UV Spectroscopy	s47	07-Feb-2022	s47
RNA Content s47 s47	UV Spectroscopy	s47	07-Feb-2022	s47

Table 2. Drug Substance Quality Control Tests

Test	Test Method	Specification	Date of Test	Result
Clarity	Appearance (Clarity)	<= 6 NTU	08-Feb-2022	<= 6 NTU
Coloration	Appearance (Coloration)	Not more intensely colored than level 7 of the brown (B) color standard	08-Feb-2022	complies
pH	Potentiometry	7.0 +/- 0.5	11-Feb-2022	7.0
Content (RNA Concentration)	UV Spectroscopy	s47	07-Feb-2022	s47
Identity of Encoded RNA Sequence	RT-PCR	Identity confirmed	15-Feb-2022	Complies
RNA Integrity	Capillary Gel Electrophoresis	s47	16-Feb-2022	s47
5'- Cap	RP-HPLC		21-Feb-2022	
Poly(A) Tail	ddPCR		15-Feb-2022	
Residual DNA Template	qPCR		20-Feb-2022	
Residual dsRNA	Immunoblot		21-Feb-2022	
Bacterial Endotoxin	Endotoxin (LAL)		08-Feb-2022	
Bioburden	Bioburden		08-Feb-2022	

Test missing - see below

Abbreviations: NTU = Nephelometric Turbidity Units; RT-PCR = Reverse Transcription PCR; dd-PCR = droplet digital PCR; IP-RP-HPLC = Ion-Pair Reversed-Phase HPLC; qPCR = quantitative PCR; dsRNA = double stranded RNA; LAL = Limulus Amebocyte Lysate; EU = Endotoxin Unit; CFU = Colony Forming Unit

Test Missing
Poly(A) Tail Length (spec - Poly(A) Tail Length Confirmed)

Added to DS release tests for Bivalent formulation in PM-2022-03551-1-2 and for the Monovalent formulation in PM-2021-04026-1-2

8.11.22 s22

All results comply

COMIRNATY Genealogy Flow

Process Stage	Batch Number	Input Process Stage	Input Batch Number(s)
Working Cell Bank	32134855	N/A	N/A
Master Cell Bank Omicron	FT1817	N/A	N/A
Linearized DNA Template	B625536	Working Cell Bank	32134855
Linearized DNA Template Omicron	FT0614	Master Cell Bank Omicron	FT1817
BNT162b2 Drug Substance	2236630_MB0084	Linearized DNA Template	B625536
BNT162b2 Omicron Drug Substance	2241554_MB0010	Linearized DNA Template Omicron	FT0614
BNT162b2 Omicron Drug Substance	2241554_MB0011	Linearized DNA Template Omicron	FT0614
LNP Fabrication and Bulk Drug Product Formulation	GE1848	BNT162b2 Drug Substance & BNT162b2 Omicron Drug Substance	2236630_MB0084, 2241554_MB0010, 2241554_MB0011
Semi-Finished Goods	GE1674	LNP Fabrication and Bulk Drug Product Formulation	GE1848
Packaged Lot	GE1643	Semi-Finished Goods	GE1674

Prepared By:

s22 s22

02 Sep 2022 03:48:049-0400

REASON: I approve this document.

9633d8dc-763c-4d96-aa2e-f081e9d901ed

Approved By:

s22 s22

02 Sep 2022 04:11:012-0400

REASON: I approve this document.

d007747d-0c63-4417-9d1e-20158a2a4d4b

Validation Report

1 ✓

Subject CN	TAIGLE LLC
Subject DN	EMAILADDRESS=operations@msbdocs.com,CN=TAIGLE LLC,OU=MSB,O=TAIGLE LLC,L=Irvine,ST=California,C=US
Email	operations@msbdocs.com
Serial #	132378441527873428230597372186267991 46
Issuer DN	CN=Entrust Class 3 Client CA - SHA256,OU=(c) 2015 Entrust, Inc. - for authorized use only,OU=See www.entrust.net/legal-terms,O=Entrust, Inc.,C=US
Signing Time	02 Sep 2022 04:11:012-0400

- ✓ The Certificate chain was successfully built to a Trusted Root Certificate.
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- ✓ The Document has not been modified since the signature was applied.

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DocUUID : 60584476-420c-43ce-9d1e-5b03ae4fb9f0

Audit Trail Report

Document Name: LRP_GE1643_20220902_SCHO.pdf

Document ID: 60584476-420c-43ce-9d1e-5b03ae4fb9f0

Time Stamp	User	Action	Details
02 Sep 2022 03:45:009-0400	s22 [redacted] UUID : d007747d-0c63-4417-9d1e-20158a2a4d4b Email : s22 [redacted]@pfizer.com IP Address : 168.224.160.14	Started	The custodian composed the ePak succesfully. Subject: LRP_GE1643_20220902_SCHO ePak UUID: e02b8e27-e8e2-4aa2-9ad9-9e0249b1bc6e
02 Sep 2022 03:45:009-0400	s22 [redacted] UUID : 9633d8dc-763c-4d96-aa2e-f081e9d901ed Email : s22 [redacted]@pfizer.com	RequestSent	Sign request sent to ePak recipient.
02 Sep 2022 03:45:009-0400	s22 [redacted] UUID : d007747d-0c63-4417-9d1e-20158a2a4d4b Email : s22 [redacted]@pfizer.com	RequestSent	Sign request sent to ePak recipient.
02 Sep 2022 03:45:011-0400	s22 [redacted] UUID : d007747d-0c63-4417-9d1e-20158a2a4d4b Email : s22 [redacted]@pfizer.com IP Address : 168.224.160.14	DocumentViewed	Document viewed by signer.
02 Sep 2022 03:47:051-0400	s22 [redacted] UUID : 9633d8dc-763c-4d96-aa2e-f081e9d901ed Email : s22 [redacted]@pfizer.com IP Address : 168.224.160.14	DocumentViewed	Document viewed by signer.
02 Sep 2022 03:48:018-0400	s22 [redacted] UUID : 9633d8dc-763c-4d96-aa2e-f081e9d901ed Email : s22 [redacted]@pfizer.com IP Address : 168.224.160.14	SignerTagFilled	The signer filled Signer Text. Value: QP Delegate
02 Sep 2022 03:48:049-0400	s22 [redacted] UUID : 9633d8dc-763c-4d96-aa2e-f081e9d901ed Email : s22 [redacted]@pfizer.com IP Address : 168.224.160.14	Signed	The recipient signed the document with no comments. Comments: None Reason: I approve this document. I approve this document. I approve this document. Consent: I understand that my Electronic Signature is Equivalent to my Handwritten Signature and is therefore legally binding. My Electronic Signature will remain unique to me, and under no circumstance I am allowed to disclose my password to any individual which may allow unauthorized access to system. I understand that I am accountable and responsible for all actions associated with my Electronic Signature.
02 Sep 2022 04:10:057-0400	s22 [redacted] UUID : d007747d-0c63-4417-9d1e-20158a2a4d4b Email s22 [redacted]@pfizer.com IP Address : 168.224.160.14	SignerTagFilled	The signer filled Signer Text. Value: QP Delegate

02 Sep 2022 04:11:012-0400	s22 [redacted] UUID : d007747d-0c63-4417-9d1e-20158a2a4d4b Email : s22 [redacted]@pfizer.com IP Address : 168.224.160.14	Signed	The recipient signed the document with no comments. Comments: None Reason: I approve this document. I approve this document. I approve this document. Consent: I understand that my Electronic Signature is Equivalent to my Handwritten Signature and is therefore legally binding. My Electronic Signature will remain unique to me, and under no circumstance I am allowed to disclose my password to any individual which may allow unauthorized access to system. I understand that I am accountable and responsible for all actions associated with my Electronic Signature.
02 Sep 2022 04:11:012-0400	s22 [redacted] UUID : d007747d-0c63-4417-9d1e-20158a2a4d4b Email : s22 [redacted]@pfizer.com	Completed	The ePak is completed successfully.

REVIEWED
By s22 at 3:15 pm, Nov 07, 2022

Rec 07NOV2022
2211003539 Prot
2211003540 Initial

Document 20

sciensano

LIMS

Contact: s22
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BELAC 081-TEST

Quality of Vaccines and Blood Products

EU OFFICIAL CONTROL AUTHORITY BATCH RELEASE CERTIFICATE FOR IMMUNOLOGICAL PRODUCTS

EU/EEA OFFICIAL CONTROL AUTHORITY BATCH RELEASE CERTIFICATE – Finished Product

Examined under Article 114 of Directive 2001/83/EC as amended by Directive 2004/27/EC (Immunological Medicinal Products) and in accordance with the Administrative Procedure for Official Control Authority Batch Release.

Trade name	**COMIRNATY Original/Omicron BA.1**
International Non-proprietary Name / Ph.Eur. name / Common name	**Pandemic COVID-19 Vaccine (mRNA)/ Bivalent 15µg/15µg (Original and Omicron BA.1)**
Batch numbers appearing on the package and other identification numbers associated with this batch[1]	**GD6794**
Type of container	**Vial**
Total number of containers in this batch	s47
Number of doses per container	**6 doses**
Date of start of period of validity	**24 May 2022**
Date of expiry	**30 April 2023**
Marketing Authorisation number (member state / EU) issued by	**EU/1/20/1528/006-007**
Name and address of manufacturer	**Pfizer Manufacturing Belgium NV / 2870 Puurs, Belgium**
Name and address of marketing authorisation holder if different	**BioNTech Manufacturing GmbH An der Goldgrube 12 55131 Mainz, Germany**

This batch has been examined using documented procedures which form part of a quality system which is in accordance with the ISO/IEC 17025 standard.
This examination is based on the relevant EU OCABR guideline for this product.

This batch is in compliance with the approved specifications laid down in the relevant European Pharmacopoeia monographs and the above marketing authorisation and is released.

Signature **Date of issue**	Digitally signed by s22 Date: 2022.09.02 12:46:33 +02'00' LIMS
Name and function of signatory	**p.o. s22** **Quality of Vaccines and Blood Products Service**

Certificate number: BE/22/1989 LIMS

[1] Such as batch number of final bulk

Sciensano Rue Juliette Wytsmanstraat 14 1050 Brussels Belgium
T + 32 2 642 51 11 F + 32 2 642 50 01 info@sciensano.be www.sciensano.be

healthy all life long

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REVIEWED
By s22 at 1:37 pm, Nov 08, 2022

Pfizer Manufacturing Belgium NV
Rijksweg 12
2870 Puurs
Belgium

Date: 23-Aug-2022

TGA Protocol Checklist used: QPulse Bio-BRU-Form-65 Version 8 8.11.22 s22

Validator - Please note anomalies (ncorrect spec & missing DS tests) as bookmarked. 8.11.22 s22

Sciensano
Quality of Vaccines and Blood Products
Juliette Wytsmanstraat 14
B1050 Brussels
Belgium

Protocol passed in LIMS (as per s22 Company to be sent a single email for all protocols currently on hand (8.11.22) outlining the anomalies which are consistent across all protocols. 8.11.22 s22

Email sent 11.11.22 D22-6083541 s22

Follow up email sent D22-6236511 19.12.22 s22

Please consider this as an official request for Official Control Authority Batch Release (OCABR) and WHO release for the following product:

Trade Name:	COMIRNATY
Type of Container:	Vial
Storage Conditions:	-90°C to -60°C
Marketing Authorisation Holder:	BioNTech Manufacturing GmbH, An der Goldgrube 12, 55131 Mainz, Germany
Batch Number:	GD6794
Presentation:	Bivalent [Original and Omicron BA.1] AUSTR 394890
Process Variation:	Adult (15/15 µg)
Date of Filling:	03-Jun-2022
Expiry Date:	30-Apr-2023
Total Quantity:*	s47
Site of Manufacture:	Pfizer Manufacturing Belgium NV, PGS Puurs, Rijksweg 12, 2870 Puurs, Belgium

No response received. However, enquiry has now been CLOSED in agreeance with s22
- Sponsor notified by email
- See D23-5484447 20.6.23 s22

*No deviations were raised in context of yield for the batch. Therefore, there were no exceedance of the yield limits

Please find enclosed the lot release protocol and genealogy diagram for this batch. The lot release protocol has been reviewed by a Qualified Person and found to be satisfactory. The OMCL performing OCABR has been notified of all relevant approved variations that have an impact on product specifications or on data supplied in section 3 of this protocol as described in the EU administrative procedure for OCABR.

In addition, certification by qualified person that the above COMIRNATY batch was manufactured following all national requirements and complies with WHO good manufacturing practices for pharmaceutical products: main principles; WHO good manufacturing practices for biological products; and WHO Guidelines for independent lot release of vaccines by regulatory authorities.

- WHO Technical Report Series, No. 986, Annex 2.
- WHO Technical Report Series, No. 999, Annex 2.
- WHO Technical Report Series, No. 978, Annex 2

Prepared By:
s22 s22
02 Sep 2022 03:47:033-0400
REASON: I approve this document.
9633d8dc-763c-4d96-aa2e-f081e9d901ed
QP Delegate

Approved By:
s22 s22
02 Sep 2022 04:12:000-0400
REASON: I approve this document.
d007747d-0c63-4417-9d1e-20158a2a4d4b
QP Delegate

DocUUID : 4a05b0d0-90fc-424a-b7cb-fe2a28254a5b

REASON FOR SUBMISSION:
For Release

Lot Number: GD6794
Trade Name of Product: COMIRNATY
Licensed Name of Product: COMIRNATY
Marketing Authorisation Holder Name and Address: BioNTech Manufacturing GmbH, An der Goldgrube 12, 55131 Mainz, Germany
Manufacturing Site: Pfizer Manufacturing Belgium NV, PGS Puurs, Rijksweg 12, 2870 Puurs, Belgium
Marketing Authorisation Number: EU/1/20/1528/006

Date of Manufacture: 24-May-2022 LIMS **Date of Expiry:** 30-Apr-2023
Date of Fill: 03-Jun-2022

Product Information:

Drug Substance Target Concentration: s47 mg/mL

LOT GENEALOGY

Component Description	Batch Number	Date of Manuf.	Manufacture Site	Quantity
Working Cell Bank	32134855	01-Jun-2021	Sandoz GmbH / Novartis, Austria	N/A
Master Cell Bank Omicron	FT1817	24-Dec-2021	Pfizer Andover	N/A
DNA Plasmid linearized	B624949	29-Sep-2021	Sandoz GmbH / Novartis, Austria	s47
DNA Plasmid linearized Omicron	FT0614	06-Jan-2022	Pfizer Andover	s47
BNT162b2 Drug Substance	2236630_MB0079 / 1086842	21-Jan-2022	BioNTech Manufacturing Marburg GmbH / Rentschler Biopharma SE	s47
BNT162b2 Omicron Drug Substance	2241554_MB0009	04-Feb-2022	BioNTech Manufacturing Marburg GmbH	s47
BNT162b2 Omicron Drug Substance	2241554_MB0010	06-Feb-2022	BioNTech Manufacturing Marburg GmbH	s47
LNP Fabrication and Bulk Drug Product Formulation*	GE8901	24-May-2022	Pfizer Puurs	s47
Semi-Finished Goods	GD7270	24-May-2022	Pfizer Puurs	s47
Drug Product Packaging	GD6794	24-May-2022	Pfizer Puurs	s47

see below

DNA Linearized Plasmid (2x lots) Not mentioned in protocol other than in lot genealogy and genealogy flowchart 8.11.22 s22

Semi-Finished Goods GD7270 Not mentioned in protocol other than in lot genealogy and genealogy flowchart 8.11.22 s22

*No LNP bulk stored as drug product intermediate was used during manufacturing.

MCB Omicron FT1817
Approved in PM-2022-03551-1-2 Evaluation D22-5836308 Approval D22-6018548

Checklist update has been flagged & added to QPulse (CR522) 8.11.22 s22

DocUUID : 4a05b0d0-00fc-424e-b7cb-fe2a26254a5b

Lot Number: GD6794

Licensed Name of Product: COMIRNATY

FILL INFORMATION

Container Type:	Vial	**Volume per container:**	2.25 mL
Approved Storage Period:	12 months	**Storage Temperature:**	-90°C to -60°C
Number of containers for release:	s47	**Number of Doses per container:**	6
Single human dose strength:	15/15 µg/Dose	**Start Date of period of Validity:**	Date of Manufacture

Certification by qualified person taking the overall responsibility for production and control of the product was manufactured and tested according to the procedures approved by the competent authorities and complies with the quality requirements. This includes that, for any materials derived from ruminants (bovine, ovine, caprine) used in the manufacture and/or formulation of the batch of product specified above, all measures have been taken to demonstrate compliance with Directive 2001/83/EC and amending Directives 2003/63/EC and 2004/27/EC.

In addition, the OMCL performing OCABR has been notified of all relevant approved variations that have an impact on product specifications or on data supplied in section 3 of this protocol as described in the EU administrative procedure for OCABR.

Prepared By:

s22 s22

02 Sep 2022 03:47:033-0400

REASON: I approve this document.

9633d8dc-763c-4d96-aa2e-f081e9d901ed

Approved By:

s22 s22

02 Sep 2022 04:12:000-0400

REASON: I approve this document.

d007747d-0c63-4417-9d1e-20158a2a4d4b

DocUUID : 4a05b0d0-06fc-424a-b7cb-fe2a26254a5b

Lot Number:GD6794

Licensed Name of Product: COMIRNATY

Table 1. **Filled Vaccine Quality Control Tests**

Test	Test Method	Specification	Date of Test	Result
Appearance	Appearance (Visual)	White to off-white suspension	22-Jun-2022	MEETS TEST
Appearance (Visible Particulates)	Appearance (Particles)	May contain white to off-white opaque amorphous particles	22-Jun-2022	MEETS TEST
Subvisible Particles	Subvisible Particulate Matter	Particles >= 10µm: <= 6000 per container	15-Jun-2022	s47
Subvisible Particles	Subvisible Particulate Matter	Particles >= 25µm: <= 600 per container	15-Jun-2022	
pH	Potentiometry	6.9 - 7.9	16-Jun-2022	7.4
Osmolality	Osmometry	s47	16-Jun-2022	s47
LNP Size	Dynamic Light Scattering (DLS)		17-Jun-2022	
LNP Polydispersity	Dynamic Light Scattering (DLS)		17-Jun-2022	
RNA Encapsulation	Fluorescence assay		15-Jun-2022	
RNA content	Fluorescence assay		15-Jun-2022	
ALC-0315 content	HPLC-CAD		16-Jun-2022	
ALC-0159 content	HPLC-CAD		16-Jun-2022	
DSPC content	HPLC-CAD		16-Jun-2022	
Cholesterol content	HPLC-CAD		16-Jun-2022	
Container content for injections	Vial Content (Volume)		-Jun-2022 see below	
Lipid identities	HPLC-CAD	Retention times consistent with references (ALC-0315, ALC-0159, Cholesterol, DSPC)	16-Jun-2022	MEETS TEST

LIMS

s47

All results comply

Lot Number: GD6794

Licensed Name of Product: COMIRNATY

Table 1 (Continued) Filled Vaccine Quality Control Tests

Test	Test Method	Specification	Date of Test	Result
Identity of encoded RNA sequence	ddPCR	Identity confirmed: BNT162b2	29-Jul-2022	Identity Confirmed
Identity of encoded RNA sequence	ddPCR	Identity confirmed: BNT162b2 Omicron	29-Jul-2022	Identity Confirmed
RNA Ratio	ddPCR	s47 BNT162b2	29-Jul-2022	s47 LIMS
RNA Ratio	ddPCR	s47 BNT162b2 Omicron	29-Jul-2022	
In Vitro Expression	Cell-based Flow Cytometry	s47	01-Jul-2022	
RNA Integrity	Capillary Gel Electrophoresis	s47	20-Jun-2022	
Bacterial Endotoxin	Endotoxin (LAL)	s47	09-Jun-2022	

Abbreviations: LNP = Lipid Nanoparticles; HPLC = High-Performance Liquid Chromatography; CAD = Charged Aerosol Detector; ddPCR = droplet digital Polymerase Chain Reaction; LAL = Limulus Amebocyte Lysate; EU = Endotoxin Unit

Filled Vaccine Quality Control Tests (cont.)

All results comply

Sterility

Method: Membrane Filtration

Type: Final Container

Container: Sterility-20 mL/medium (40 vials)

Date On Test	Medium/Temperature	Date Off Test	Specification	Test Result
08-Jun-2022	Thioglycollate 30°C-35°C	23-Jun-2022	No growth observed	No growth observed
	Soybean Casein Digest 20°C-25°C		No growth observed	No growth observed

DocUUID : 4a05b0d0-00fc-424a-b7cb-fe2a26254a5b

Lot Number: GD6794

Licensed Name of Product: COMIRNATY

Table 2: Fill Vaccine In-Process Tests – Fill Weight Measurements

Pump	Minimum	Maximum	Mean
1	s47		
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			

Acceptance Criteria: s47
All measurements were within limits.

Lot Number: GD6794
Licensed Name of Product: COMIRNATY

BNT162b2 LNP Fabrication

LNP Lot Number: GE8901

Table 1: In-Process Tests (IPT-C)

Test	Test Method	Specification	Date of Test	Result
pH s47	Potentiometry	s47	29-May-2022	s47
pH s47	Potentiometry		29-May-2022	
RNA Content s47 s47 s47 s4	Fluorescence assay		02-Jun-2022	

Lot Number: GD6794

Licensed Name of Product: COMIRNATY

BNT162b2 Drug Substance

Lot Number: 2236630_MB0079

Date of Manufacture: 21-Jan-2022 ✓ **Date of Expiry:** 30-Jun-2022 ✓

Storage Temperature: - 25°C to - 15°C **Approved Storage Period:** 6 months

Consumed Quantity: s47

Table 1. Drug Substance In-Process Tests (IPT-C)

Test	Test Method	Specification ✓	Date of Test	Result ✓
RNA Content s47 s47	UV Spectroscopy	s47	21-Jan-2022	s47
RNA Content s47 s47	UV Spectroscopy	s47	21-Jan-2022	s47

Table 2. Drug Substance Quality Control Tests

Test	Test Method	Specification ✓	Date of Test	Result ✓
Clarity	Appearance (Clarity)	<= 6 NTU	25-Jan-2022	0 NTU
Coloration	Appearance (Coloration)	Not more intensely colored than level 7 of the brown (B) color standard	25-Jan-2022	complies
pH	Potentiometry	7.0 +/- 0.5	25-Jan-2022	7.0
Content (RNA Concentration)	UV Spectroscopy	s47	09-Mar-2022	s47
Identity of Encoded RNA Sequence	RT-PCR	Identity confirmed	26-Jan-2022	Complies
RNA Integrity	Capillary Gel Electrophoresis	s47	08-Feb-2022	s47
5'- Cap	RP-HPLC		10-Feb-2022	
Poly(A) Tail	ddPCR		09-Feb-2022	
Residual DNA Template	qPCR		07-Feb-2022	
Residual dsRNA	Immunoblot		10-Feb-2022	
Bacterial Endotoxin	Endotoxin (LAL)		24-Jan-2022	
Bioburden	Bioburden		28-Jan-2022	

Test missing - see below

Abbreviations: NTU = Nephelometric Turbidity Units; RT-PCR = Reverse Transcription PCR; dd-PCR = droplet digital PCR; IP-RP-HPLC = Ion-Pair Reversed-Phase HPLC; qPCR = quantitative PCR; dsRNA = double stranded RNA; LAL = Limulus Amebocyte Lysate; EU = Endotoxin Unit; CFU = Colony Forming Unit

Test Missing
Poly(A) Tail Length (spec - Poly(A) Tail Length Confirmed)

Added to DS release tests for Bivalent formulation in PM-2022-03551-1-2 and for the Monovalent formulation in PM-2021-04026-1-2

8.11.22 s22

All results comply

DocUUID : 4a05b0d0-00fc-424a-b7cb-fe2a26254a5b

Lot Number: GD6794

Licensed Name of Product: COMIRNATY

BNT162b2 Omicron Drug Substance

Lot Number: 2241554_MB0009

Date of Manufacture: 04-Feb-2022 ✓ **Date of Expiry:** 20-Jul-2022 ✓

Storage Temperature: - 25°C to - 15°C **Approved Storage Period:** 6 months

Consumed Quantity: s47

Table 1. Drug Substance In-Process Tests (IPT-C)

Test	Test Method	Specification ✓	Date of Test	Result
RNA Content s47 s47	UV Spectroscopy	s47	04-Feb-2022	s47
RNA Content s47 s47	UV Spectroscopy	s47	04-Feb-2022	s47

Table 2. Drug Substance Quality Control Tests

Test	Test Method	Specification ✓	Date of Test	Result ✓
Clarity	Appearance (Clarity)	<= 6 NTU	08-Feb-2022	<= 6 NTU
Coloration	Appearance (Coloration)	Not more intensely colored than level 7 of the brown (B) color standard	08-Feb-2022	complies
pH	Potentiometry	7.0 +/- 0.5	07-Feb-2022	7.0
Content (RNA Concentration)	UV Spectroscopy	s47	04-Feb-2022	s47
Identity of Encoded RNA Sequence	RT-PCR	Identity confirmed	15-Feb-2022	Complies
RNA Integrity	Capillary Gel Electrophoresis	s47	08-Feb-2022	s47
5'- Cap	RP-HPLC		10-Feb-2022	
Poly(A) Tail	ddPCR		15-Feb-2022	
Residual DNA Template	qPCR		09-Feb-2022	
Residual dsRNA	Immunoblot		14-Feb-2022	
Bacterial Endotoxin	Endotoxin (LAL)		04-Feb-2022	
Bioburden	Bioburden		04-Feb-2022	

Test missing - see below

Abbreviations: NTU = Nephelometric Turbidity Units; RT-PCR = Reverse Transcription PCR; dd-PCR = droplet digital PCR; IP-RP-HPLC = Ion-Pair Reversed-Phase HPLC; qPCR = quantitative PCR; dsRNA = double stranded RNA; LAL = Limulus Amebocyte Lysate; EU = Endotoxin Unit; CFU = Colony Forming Unit

Test Missing
Poly(A) Tail Length (spec - Poly(A) Tail Length Confirmed)

Added to DS release tests for Bivalent formulation in PM-2022-03551-1-2 and for the Monovalent formulation in PM-2021-04026-1-2

8.11.22 s22

All results comply

Lot Number: GD6794

Licensed Name of Product: COMIRNATY

BNT162b2 Omicron Drug Substance

Lot Number: 2241554_MB0010

Date of Manufacture: 06-Feb-2022 ✓ **Date of Expiry:** 04-Aug-2022 ✓

Storage Temperature: - 25°C to - 15°C **Approved Storage Period:** 6 months

Consumed Quantity: s47

Table 1. Drug Substance In-Process Tests (IPT-C)

Test	Test Method	Specification ✓	Date of Test	Result ✓
RNA Content s47 s47	UV Spectroscopy	s47	05-Feb-2022	s47
RNA Content s47 s47	UV Spectroscopy	s47	05-Feb-2022	s47

Table 2. Drug Substance Quality Control Tests

Test	Test Method	Specification ✓	Date of Test	Result ✓
Clarity	Appearance (Clarity)	<= 6 NTU	08-Feb-2022	<= 6 NTU
Coloration	Appearance (Coloration)	Not more intensely colored than level 7 of the brown (B) color standard	08-Feb-2022	complies
pH	Potentiometry	7.0 +/- 0.5	07-Feb-2022	7.0
Content (RNA Concentration)	UV Spectroscopy	s47	05-Feb-2022	s47
Identity of Encoded RNA Sequence	RT-PCR	Identity confirmed	15-Feb-2022	Complies
RNA Integrity	Capillary Gel Electrophoresis	s47	08-Feb-2022	s47
5'- Cap	RP-HPLC		10-Feb-2022	
Poly(A) Tail	ddPCR		15-Feb-2022	
Residual DNA Template	qPCR		09-Feb-2022	
Residual dsRNA	Immunoblot		14-Feb-2022	
Bacterial Endotoxin	Endotoxin (LAL)		08-Feb-2022	
Bioburden	Bioburden		08-Feb-2022	

Test missing - see below

Abbreviations: NTU = Nephelometric Turbidity Units; RT-PCR = Reverse Transcription PCR; dd-PCR = droplet digital PCR; IP-RP-HPLC = Ion-Pair Reversed-Phase HPLC; qPCR = quantitative PCR; dsRNA = double stranded RNA; LAL = Limulus Amebocyte Lysate; EU = Endotoxin Unit; CFU = Colony Forming Unit

Test Missing
Poly(A) Tail Length (spec - Poly(A) Tail Length Confirmed)

Added to DS release tests for Bivalent formulation in PM-2022-03551-1-2 and for the Monovalent formulation in PM-2021-04026-1-2

8.11.22 s22

All results comply

DocUUID : 4a05b0d0-00fc-424a-b7cb-fe2a26254a5b

COMIRNATY Genealogy Flow

Process Stage	Batch Number	Input Process Stage	Input Batch Number(s)
Working Cell Bank	32134855	N/A	N/A
Master Cell Bank Omicron	FT1817	N/A	N/A
Linearized DNA Template	B624949	Working Cell Bank	32134855
Linearized DNA Template Omicron	FT0614	Master Cell Bank Omicron	FT1817
BNT162b2 Drug Substance	2236630_MB0079	Linearized DNA Template	B624949
BNT162b2 Omicron Drug Substance	2241554_MB0009	Linearized DNA Template Omicron	FT0614
BNT162b2 Omicron Drug Substance	2241554_MB0010	Linearized DNA Template Omicron	FT0614
LNP Fabrication and Bulk Drug Product Formulation	GE8901	BNT162b2 Drug Substance & BNT162b2 Omicron Drug Substance	2236630_MB0079, 2241554_MB0009, 2241554_MB0010
Semi-Finished Goods	GD7270	LNP Fabrication and Bulk Drug Product Formulation	GE8901
Packaged Lot	GD6794	Semi-Finished Goods	GD7270

Prepared By:

s22 s22

02 Sep 2022 03:47:033-0400

REASON: I approve this document.

9633d8dc-763c-4d96-aa2e-f081e9d901ed

Approved By:

s22 s22

02 Sep 2022 04:12:000-0400

REASON: I approve this document.

d007747d-0c63-4417-9d1e-20158a2a4d4b

DocUUID : 4a05b0d0-00fc-424a-b7cb-fe2a26254a5b

Validation Report

1

Subject CN	TAIGLE LLC
Subject DN	EMAILADDRESS=operations@msbdocs.com,CN=TAIGLE LLC,OU=MSB,O=TAIGLE LLC,L=Irvine,ST=California,C=US
Email	operations@msbdocs.com
Serial #	132378441527873428230597372186267991 46
Issuer DN	CN=Entrust Class 3 Client CA - SHA256,OU=(c) 2015 Entrust, Inc. - for authorized use only,OU=See www.entrust.net/legal-terms,O=Entrust, Inc.,C=US
Signing Time	02 Sep 2022 04:12:000-0400

- The Certificate chain was successfully built to a Trusted Root Certificate.
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Audit Trail Report

Document Name: LRP_GD6794_20220902_SCHO.pdf

Document ID: 4a05b0d0-00fc-424a-b7cb-fe2a26254a5b

Time Stamp	User	Action	Details
02 Sep 2022 03:38:032-0400	s22 [redacted] UUID : d007747d-0c63-4417-9d1e-20158a2a4d4b Email : s22 [redacted]@pfizer.com IP Address : 168.224.160.14	Started	The custodian composed the ePak succesfully. Subject: LRP_GD6794_20220902_SCHO ePak UUID: 6cec70d6-493a-49f3-99fa-6215d68f683a
02 Sep 2022 03:38:032-0400	s22 [redacted] UUID : 9633d8dc-763c-4d96-aa2e-f081e9d901ed Email : s22 [redacted]@pfizer.com	RequestSent	Sign request sent to ePak recipient.
02 Sep 2022 03:38:032-0400	s22 [redacted] UUID : d007747d-0c63-4417-9d1e-20158a2a4d4b Email : s22 [redacted]@pfizer.com	RequestSent	Sign request sent to ePak recipient.
02 Sep 2022 03:38:034-0400	s22 [redacted] UUID : d007747d-0c63-4417-9d1e-20158a2a4d4b Email : s22 [redacted]@pfizer.com IP Address : 168.224.160.14	DocumentViewed	Document viewed by signer.
02 Sep 2022 03:46:021-0400	s22 [redacted] UUID : 9633d8dc-763c-4d96-aa2e-f081e9d901ed Email : s22 [redacted]@pfizer.com IP Address : 168.224.160.14	DocumentViewed	Document viewed by signer.
02 Sep 2022 03:46:048-0400	s22 [redacted] UUID : 9633d8dc-763c-4d96-aa2e-f081e9d901ed Email : s22 [redacted]@pfizer.com IP Address : 168.224.160.14	SignerTagFilled	The signer filled Signer Text. Value: QP Delegate
02 Sep 2022 03:47:033-0400	s22 [redacted] UUID : 9633d8dc-763c-4d96-aa2e-f081e9d901ed Email : s22 [redacted]@pfizer.com IP Address : 168.224.160.14	Signed	The recipient signed the document with no comments. Comments: None Reason: I approve this document. I approve this document. I approve this document. Consent: I understand that my Electronic Signature is Equivalent to my Handwritten Signature and is therefore legally binding. My Electronic Signature will remain unique to me, and under no circumstance I am allowed to disclose my password to any individual which may allow unauthorized access to system. I understand that I am accountable and responsible for all actions associated with my Electronic Signature.
02 Sep 2022 04:11:028-0400	s22 [redacted] UUID : d007747d-0c63-4417-9d1e-20158a2a4d4b Email : s22 [redacted]@pfizer.com IP Address : 168.224.160.14	SignerTagFilled	The signer filled Signer Text. Value: QP Delegate

02 Sep 2022 04:12:000-0400	s22 [redacted] UUID : d007747d-0c63-4417-9d1e-20158a2a4d4b Email : s22 [redacted]@pfizer.com IP Address : 168.224.160.14	Signed	The recipient signed the document with no comments. Comments: None Reason: I approve this document. I approve this document. I approve this document. Consent: I understand that my Electronic Signature is Equivalent to my Handwritten Signature and is therefore legally binding. My Electronic Signature will remain unique to me, and under no circumstance I am allowed to disclose my password to any individual which may allow unauthorized access to system. I understand that I am accountable and responsible for all actions associated with my Electronic Signature.
02 Sep 2022 04:12:000-0400	s22 [redacted] UUID : d007747d-0c63-4417-9d1e-20158a2a4d4b Email : s22 [redacted]@pfizer.com	Completed	The ePak is completed successfully.

REVIEWED
By s22 at 3:56 pm, Nov 07, 2022

Rec 07NOV2022
2211003543 Prot
2211003544 Initial

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LIMS

CONTROL LABORATORY · NATIONAL · BELGIUM

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BELAC 081-TEST

Contact: s22
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Quality of Vaccines and Blood Products

EU OFFICIAL CONTROL AUTHORITY BATCH RELEASE CERTIFICATE FOR IMMUNOLOGICAL PRODUCTS

EU/EEA OFFICIAL CONTROL AUTHORITY BATCH RELEASE CERTIFICATE – Finished Product

Examined under Article 114 of Directive 2001/83/EC as amended by Directive 2004/27/EC (Immunological Medicinal Products) and in accordance with the Administrative Procedure for Official Control Authority Batch Release.

Trade name	**COMIRNATY Original/Omicron BA.1** ✓
International Non-proprietary Name / Ph.Eur. name / Common name	**Pandemic COVID-19 Vaccine (mRNA)/ Bivalent 15µg/15µg (Original and Omicron BA.1)**
Batch numbers appearing on the package and other identification numbers associated with this batch[1]	**GE8382** ✓
Type of container	**Vial**
Total number of containers in this batch	s47
Number of doses per container	**6 doses**
Date of start of period of validity	**01 June 2022**
Date of expiry	**31 May 2023** ✓
Marketing Authorisation number (member state / EU) issued by	**EU/1/20/1528/006-007**
Name and address of manufacturer	**Pfizer Manufacturing Belgium NV / 2870 Puurs, Belgium**
Name and address of marketing authorisation holder if different	**BioNTech Manufacturing GmbH An der Goldgrube 12 55131 Mainz, Germany**

This batch has been examined using documented procedures which form part of a quality system which is in accordance with the ISO/IEC 17025 standard.
This examination is based on the relevant EU OCABR guideline for this product.

This batch is in compliance with the approved specifications laid down in the relevant European Pharmacopoeia monographs and the above marketing authorisation and is released.

Signature **Date of issue**	Digitally signed by s22 Date: 2022.09.23 11:05:21 +02'00' LIMS
Name and function of signatory	**p.o.** s22 **Quality of Vaccines and Blood Products Service**

Certificate number: BE/22/2229 LIMS

[1] Such as batch number of final bulk

Sciensano Rue Juliette Wytsmanstraat 14 1050 Brussels Belgium
T + 32 2 642 51 11 F + 32 2 642 50 01 info@sciensano.be www.sciensano.be

.be

REVIEWED
By s22 at 2:35 pm, Nov 08, 2022

Pfizer Manufacturing Belgium NV
PGS Puurs
Rijksweg 12
2870 Puurs

Date: 01-Sep-2022

TGA Protocol Checklist used: QPulse Bio-BRU-Form-65 Version 8 8.11.22 s22

Validator - Please note anomalies (incorrect spec & missing DS tests) as bookmarked. 8.11.22 s22

Sciensano
Quality of Vaccines and Blood Products
Juliette Wytsmanstraat 14
B1050 Brussels
Belgium

Protocol passed in LIMS (as per s22 Company to be sent a single email for all protocols currently on hand (8.11.22) outlining the anomalies which are consistent across all protocols. 8.11.22 s22

Email sent 11.11.22 D22-6083541 s22

Please consider this as an official request for Official Control Authority Batch Release (OCABR) and W release for the following product:

Follow up email sent D22-6236511 19.12.22 s22

Trade Name:	COMIRNATY
Type of Container:	Vial
Storage Conditions:	-90˚C to -60˚C
Marketing Authorisation Holder:	BioNTech Manufacturing GmbH, An der Goldgrube 12, 55131 Main Germany
Batch Number:	GE8382
Presentation:	Bivalent [Original and Omicron BA.1] AUSTR 394890
Process Variation:	Adult (15/15 µg)
Date of Filling:	12-Jun-2022
Expiry Date:	31-May-2023
Total Quantity:*	s47
Site of Manufacture:	Pfizer Manufacturing Belgium NV, PGS Puurs, Rijksweg 12, 2870 Puurs, Belgium

No response received. However, enquiry has now been CLOSED in agreeance with s22 - Sponsor notified by email - See D23-5484447 20.6.23 s22

*No deviations were raised in context of yield for the batch. Therefore, there were no exceedance of the yield limits

Please find enclosed the lot release protocol and genealogy diagram for this batch. The lot release protocol has been reviewed by a Qualified Person and found to be satisfactory. The OMCL performing OCABR has been notified of all relevant approved variations that have an impact on product specifications or on data supplied in section 3 of this protocol as described in the EU administrative procedure for OCABR.

In addition, certification by qualified person that the above COMIRNATY batch was manufactured following all national requirements and complies with WHO good manufacturing practices for pharmaceutical products: main principles; WHO good manufacturing practices for biological products; and WHO Guidelines for independent lot release of vaccines by regulatory authorities.

- WHO Technical Report Series, No. 986, Annex 2.
- WHO Technical Report Series, No. 999, Annex 2.
- WHO Technical Report Series, No. 978, Annex 2

Prepared By:
s22 s22
22 Sep 2022 17:46:054-0400
REASON: I approve this document.
d007747d-0c63-4417-9d1e-20158a2a4d4b
QP Delegate

Approved By:
s22 s22
23 Sep 2022 02:03:029-0400
REASON: I approve this document.
27b6bf54-6923-48a1-b8b7-97c9993c864f
QP Delegate

DocUUID : b5bdb1ea-944c-420c-b8f4-ac816b46ad75

REASON FOR SUBMISSION:
For Release

Lot Number: GE8382
Trade Name of Product: COMIRNATY
Licensed Name of Product: COMIRNATY
Marketing Authorisation Holder Name and Address: BioNTech Manufacturing GmbH, An der Goldgrube 12, 55131 Mainz, Germany
Manufacturing Site: Pfizer Manufacturing Belgium NV, PGS Puurs, Rijksweg 12, 2870 Puurs, Belgium
Marketing Authorisation Number: EU/1/20/1528/006

Date of Manufacture: 01-Jun-2022 LIMS **Date of Expiry:** 31-May-2023
Date of Fill: 12-Jun-2022

Product Information:

Drug Substance Target Concentration: s47 [redacted] ng/mL

LOT GENEALOGY

Component Description	Batch Number	Date of Manuf.	Manufacture Site	Quantity
Working Cell Bank	DW8970	07-May-2020	Pfizer Chesterfield	N/A
Master Cell Bank Omicron	FT1817	24-Dec-2021	Pfizer Andover	N/A
DNA Plasmid linearized	FJ8256	20-Aug-2021	Pfizer Andover	s47 [redacted]
DNA Plasmid linearized	FJ9482	24-Aug-2021	Pfizer Andover	
DNA Plasmid linearized Omicron	FT0614	06-Jan-2022	Pfizer Andover	
BNT162b2 Omicron Drug Substance	2241554_MB0011	07-Feb-2022	BioNTech Manufacturing Marburg GmbH	
BNT162b2 Omicron Drug Substance	2241554_MB0016	17-Feb-2022	BioNTech Manufacturing Marburg GmbH	
BNT162b2 Drug Substance	FR6462	15-Dec-2021	Pfizer Andover	
BNT162b2 Drug Substance	FT3266	01-Jan-2022	Pfizer Andover	
LNP Fabrication and Bulk Drug Product Formulation*	GE5848	01-Jun-2022	Pfizer Puurs	
Semi-Finished Goods	GE8400	01-Jun-2022	Pfizer Puurs	
Drug Product Packaging	GE8382	01-Jun-2022	Pfizer Puurs	

see below

DNA Linearized Plasmid (3x lots) Not mentioned in protocol other than in lot genealogy and genealogy flowchart 8.11.22 s22

Semi-Finished Goods - Not mentioned in protocol other than in lot genealogy and genealogy flowchart 8.11.2 s22

*No LNP bulk stored as drug product intermediate was used during manufacturing

MCB Omicron FT1817
Approved in PM-2022-03551-1-2 Evaluation D22-5836308 Approval D22-6018548

Checklist update has been flagged & added to QPulse (CR522) 8.11.22 s22

DocUUID : b5bcb1ea-944c-420c-b9f4-ac816b45ad75

Lot Number: GE8382

Licensed Name of Product: COMIRNATY

FILL INFORMATION

Container Type:	Vial	**Volume per container:**	2.25 mL
Approved Storage Period:	12 months	**Storage Temperature:**	-90°C to -60°C
Number of containers for release:	s47 [redacted]	**Number of Doses per container:**	6
Single human dose strength:	15/15 µg/Dose	**Start Date of period of Validity:**	Date of Manufacture

Certification by qualified person taking the overall responsibility for production and control of the product was manufactured and tested according to the procedures approved by the competent authorities and complies with the quality requirements. This includes that, for any materials derived from ruminants (bovine, ovine, caprine) used in the manufacture and/or formulation of the batch of product specified above, all measures have been taken to demonstrate compliance with Directive 2001/83/EC and amending Directives 2003/63/EC and 2004/27/EC.

In addition, the OMCL performing OCABR has been notified of all relevant approved variations that have an impact on product specifications or on data supplied in section 3 of this protocol as described in the EU administrative procedure for OCABR.

Prepared By:

s22 [redacted] s22 [redacted]

22 Sep 2022 17:46:054-0400

REASON: I approve this document.

d007747d-0c63-4417-9d1e-20158a2a4d4b

Approved By:

s22 [redacted] s22 [redacted]

23 Sep 2022 02:03:029-0400

REASON: I approve this document.

27b6bf54-6923-48a1-b9b7-97c9993c864f

Lot Number:GE8382

Licensed Name of Product: COMIRNATY

Table 1. **Filled Vaccine Quality Control Tests**

Test	Test Method	Specification	Date of Test	Result
Appearance	Appearance (Visual)	White to off-white suspension	01-Jul-2022	MEETS TEST
Appearance (Visible Particulates)	Appearance (Particles)	May contain white to off-white opaque amorphous particles	01-Jul-2022	MEETS TEST
Subvisible Particles	Subvisible Particulate Matter	Particles >= 10µm: <= 6000 per container	23-Jun-2022	s47 [redacted]
Subvisible Particles	Subvisible Particulate Matter	Particles >= 25µm: <= 600 per container	23-Jun-2022	
pH	Potentiometry	6.9 - 7.9	22-Jun-2022	7.5
Osmolality	Osmometry	s47 [redacted]	28-Jun-2022	s47 [redacted]
LNP Size	Dynamic Light Scattering (DLS)		23-Jun-2022	
LNP Polydispersity	Dynamic Light Scattering (DLS)		23-Jun-2022	
RNA Encapsulation	Fluorescence assay		24-Jun-2022	
RNA content	Fluorescence assay		24-Jun-2022	
ALC-0315 content	HPLC-CAD		24-Jun-2022	
ALC-0159 content	HPLC-CAD		24-Jun-2022	
DSPC content	HPLC-CAD		24-Jun-2022	
Cholesterol content	HPLC-CAD		24-Jun-2022	
Container content for injections	Vial Content (Volume)		29-Jun-2022	
Lipid identities	HPLC-CAD	Retention times consistent with references (ALC-0315, ALC-0159, Cholesterol, DSPC)	see below 24-Jun-2022	MEETS TEST

LIMS

s47 [redacted]

All results comply

Lot Number: GE8382

Licensed Name of Product: COMIRNATY

Table 1 (Continued) Filled Vaccine Quality Control Tests

Test	Test Method	Specification	Date of Test	Result
Identity of encoded RNA sequence	ddPCR	Identity confirmed: BNT162b2	19-Aug-2022	Identity Confirmed
Identity of encoded RNA sequence	ddPCR	Identity confirmed: BNT162b2 Omicron	19-Aug-2022	Identity Confirmed
RNA Ratio	ddPCR	s47 BNT162b2	19-Aug-2022	s47
RNA Ratio	ddPCR	s47 BNT162b2 Omicron	19-Aug-2022	
In Vitro Expression	Cell-based Flow Cytometry	s47	01-Jul-2022	
RNA Integrity	Capillary Gel Electrophoresis	s47	28-Jun-2022	
Bacterial Endotoxin	Endotoxin (LAL)	s47	20-Jun-2022	

LIMS

Abbreviations: LNP = Lipid Nanoparticles; HPLC = High-Performance Liquid Chromatography; CAD = Charged Aerosol Detector; ddPCR = droplet digital Polymerase Chain Reaction; LAL = Limulus Amebocyte Lysate; EU = Endotoxin Unit

Filled Vaccine Quality Control Tests (cont.)

All results comply

Sterility

Method: Membrane Filtration

Type: Final Container

Container: Sterility-20 mL/medium (40 vials)

Date On Test	Medium/Temperature	Date Off Test	Specification	Test Result
15-Jun-2022	Thioglycollate 30°C-35°C	30-Jun-2022	No growth observed	No growth observed
	Soybean Casein Digest 20°C-25°C		No growth observed	No growth observed

Lot Number: GE8382

Licensed Name of Product: COMIRNATY

Table 2: Fill Vaccine In-Process Tests – Fill Weight Measurements

Pump	Minimum	Maximum	Mean
1	s47 [redacted]		
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			

Acceptance Criteria: s47 [redacted]g
At least one value below limits was obtained where after corrective actions were taken as per Standard Operation Procedure (SOP) and vials were rejected during IPC Fill weight.

Lot Number: GE8382
Licensed Name of Product: COMIRNATY

BNT162b2 LNP Fabrication

LNP Lot Number: GE5848

Table 1: In-Process Tests (IPT-C)

Test	Test Method	Specification	Date of Test	Result
pH s47 [redacted]	Potentiometry	s47 [redacted]	09-Jun-2022	s47 [redacted]
pH s47 [redacted]	Potentiometry		10-Jun-2022	
RNA Content s47 s47 [redacted]	Fluorescence assay		17-Jun-2022	

Lot Number: GE8382

Licensed Name of Product: COMIRNATY

BNT162b2 Omicron Drug Substance

Lot Number: 2241554_MB0011

Date of Manufacture: 07-Feb-2022 ✓ **Date of Expiry:** 07-Aug-2022 ✓

Storage Temperature: - 25°C to - 15°C **Approved Storage Period:** 6 months

Consumed Quantity: s47 [redacted]

Table 1. Drug Substance In-Process Tests (IPT-C)

Test	Test Method	Specification ✓	Date of Test	Result ✓
RNA Content s47 [redacted] s47	UV Spectroscopy	s47 [redacted]		
RNA Content s47 [redacted] s47	UV Spectroscopy			

Table 2. Drug Substance Quality Control Tests

Test	Test Method	Specification ✓	Date of Test	Result ✓
Clarity	Appearance (Clarity)	<= 6 NTU	08-Feb-2022	<= 6 NTU
Coloration	Appearance (Coloration)	Not more intensely colored than level 7 of the brown (B) color standard	08-Feb-2022	complies
pH	Potentiometry	7.0 +/- 0.5	11-Feb-2022	7.0
Content (RNA Concentration)	UV Spectroscopy	s47 [redacted]	07-Feb-2022	s47 [redacted]
Identity of Encoded RNA Sequence	RT-PCR	Identity confirmed	15-Feb-2022	Complies
RNA Integrity	Capillary Gel Electrophoresis	s47 [redacted]	16-Feb-2022	s47 [redacted]
5'- Cap	RP-HPLC		21-Feb-2022	
Poly(A) Tail	ddPCR		15-Feb-2022	
Residual DNA Template	qPCR		20-Feb-2022	
Residual dsRNA	Immunoblot		21-Feb-2022	
Bacterial Endotoxin	Endotoxin (LAL)		08-Feb-2022	
Bioburden	Bioburden		08-Feb-2022	

Test missing - see below

Abbreviations: NTU = Nephelometric Turbidity Units; RT-PCR = Reverse Transcription PCR; dd-PCR = droplet digital PCR; IP-RP-HPLC = Ion-Pair Reversed-Phase HPLC; qPCR = quantitative PCR; dsRNA = double stranded RNA; LAL = Limulus Amebocyte Lysate; EU = Endotoxin Unit; CFU = Colony Forming Unit

Test Missing
Poly(A) Tail Length (spec - Poly(A) Tail Length Confirmed)

Added to DS release tests for Bivalent formulation in PM-2022-03551-1-2 and for the Monovalent formulation in PM-2021-04026-1-2

8.11.22 s22 [redacted]

All results comply

Lot Number: GE8382

Licensed Name of Product: COMIRNATY

BNT162b2 Omicron Drug Substance

Lot Number: 2241554_MB0016

Date of Manufacture: 17-Feb-2022 ✓ **Date of Expiry:** 31-Jul-2022 ✓

Storage Temperature: - 25°C to - 15°C **Approved Storage Period:** 6 months

Consumed Quantity: s47 [redacted]

Table 1. Drug Substance In-Process Tests (IPT-C)

Test ✓	Test Method	Specification	Date of Test	Result
RNA Content s47 [redacted]	UV Spectroscopy	s47 [redacted]	17-Feb-2022	s47 [redacted]
RNA Content s47 [redacted]	UV Spectroscopy		17-Feb-2022	

Table 2. Drug Substance Quality Control Tests

Test ✓	Test Method	Specification	Date of Test	Result
Clarity	Appearance (Clarity)	<= 6 NTU	18-Feb-2022	<= 6 NTU
Coloration	Appearance (Coloration)	Not more intensely colored than level 7 of the brown (B) color standard	18-Feb-2022	complies
pH	Potentiometry	7.0 +/- 0.5	21-Feb-2022	7.0
Content (RNA Concentration)	UV Spectroscopy	s47 [redacted]	17-Feb-2022	s47 [redacted]
Identity of Encoded RNA Sequence	RT-PCR	Identity confirmed	08-Mar-2022	Complies
RNA Integrity	Capillary Gel Electrophoresis	s47 [redacted]	24-Feb-2022	s47 [redacted]
5'- Cap	RP-HPLC		28-Feb-2022	
Poly(A) Tail	ddPCR		28-Feb-2022	
Residual DNA Template	qPCR		09-Mar-2022	
Residual dsRNA	Immunoblot		24-Feb-2022	
Bacterial Endotoxin	Endotoxin (LAL)		18-Feb-2022	
Bioburden	Bioburden		18-Feb-2022	

Test missing - see below

Abbreviations: NTU = Nephelometric Turbidity Units; RT-PCR = Reverse Transcription PCR; dd-PCR = droplet digital PCR; IP-RP-HPLC = Ion-Pair Reversed-Phase HPLC; qPCR = quantitative PCR; dsRNA = double stranded RNA; LAL = Limulus Amebocyte Lysate; EU = Endotoxin Unit; CFU = Colony Forming Unit

Test Missing
Poly(A) Tail Length (spec - Poly(A) Tail Length Confirmed)

Added to DS release tests for Bivalent formulation in PM-2022-03551-1-2 and for the Monovalent formulation in PM-2021-04026-1-2

8.11.22 s22 [redacted]

All results comply

Lot Number: GE8382

Licensed Name of Product: COMIRNATY

BNT162b2 Drug Substance

Lot Number: FR6462

Date of Manufacture: 15-Dec-2021 ✓ **Date of Expiry:** 17-Aug-2022 ✓

Storage Temperature: - 25°C to - 15°C **Approved Storage Period:** 6 months

Consumed Quantity: s47 [redacted]

Table 1. Drug Substance In-Process Tests (IPT-C)

Test	Test Method	Specification ✓	Date of Test	Result ✓
RNA Content s47 [redacted]	UV Spectroscopy	s47 [redacted]	14-Dec-2021	s47 [redacted]
RNA Content s47 [redacted]	UV Spectroscopy		14-Dec-2021	

Table 2. Drug Substance Quality Control Tests

Test	Test Method	Specification ✓	Date of Test	Result ✓
Clarity	Appearance (Clarity)	<= 6 NTU	21-Dec-2021	<1 NTU
Coloration	Appearance (Coloration)	Not more intensely colored than level 7 of the brown (B) color standard	21-Dec-2021	</=B9/Colorless
pH	Potentiometry	7.0 +/- 0.5	17-Dec-2021	6.8
Content (RNA Concentration)	UV Spectroscopy	s47 [redacted]	20-Dec-2021	s47 [redacted]
Identity of Encoded RNA Sequence	RT-PCR	Identity confirmed	17-Dec-2021	Identity Confirmed
RNA Integrity	Capillary Gel Electrophoresis	s47 [redacted]	23-Dec-2021	s47 [redacted]
5'- Cap	RP-HPLC		22-Dec-2021	
Poly(A) Tail	ddPCR		22-Dec-2021	
Residual DNA Template	qPCR		21-Dec-2021	
Residual dsRNA	Immunoblot		29-Dec-2021	
Bacterial Endotoxin	Endotoxin (LAL)		16-Dec-2021	
Bioburden	Bioburden		23-Dec-2021	

Test missing - see below

Abbreviations: NTU = Nephelometric Turbidity Units; RT-PCR = Reverse Transcription PCR; dd-PCR = droplet digital PCR; IP-RP-HPLC = Ion-Pair Reversed-Phase HPLC; qPCR = quantitative PCR; dsRNA = double stranded RNA; LAL = Limulus Amebocyte Lysate; EU = Endotoxin Unit; CFU = Colony Forming Unit

Test Missing
Poly(A) Tail Length (spec - Poly(A) Tail Length Confirmed)

Added to DS release tests for Bivalent formulation in PM-2022-03551-1-2 and for the Monovalent formulation in PM-2021-04026-1-2

8.11.22 s22 [redacted]

All results comply

Lot Number: GE8382

Licensed Name of Product: COMIRNATY

BNT162b2 Drug Substance

Lot Number: FT3266

Date of Manufacture: 01-Jan-2022 ✓ **Date of Expiry:** 15-Jun-2022 ✓

Storage Temperature: - 25°C to - 15°C **Approved Storage Period:** 6 months

Consumed Quantity: s47 [redacted]

Table 1. Drug Substance In-Process Tests (IPT-C)

Test	Test Method	Specification ✓	Date of Test	Result ✓
RNA Content s47 [redacted]	UV Spectroscopy	s47 [redacted]	31-Dec-2021	s47 [redacted]
RNA Content s47 [redacted]	UV Spectroscopy		31-Dec-2021	

Table 2. Drug Substance Quality Control Tests

Test	Test Method	Specification ✓	Date of Test	Result ✓
Clarity	Appearance (Clarity)	<= 6 NTU	04-Jan-2022	1 NTU
Coloration	Appearance (Coloration)	Not more intensely colored than level 7 of the brown (B) color standard	04-Jan-2022	</=B9/Colorless
pH	Potentiometry	7.0 +/- 0.5	07-Jan-2022	6.8
Content (RNA Concentration)	UV Spectroscopy	s47 [redacted]	05-Jan-2022	s47 [redacted]
Identity of Encoded RNA Sequence	RT-PCR	Identity confirmed	05-Jan-2022	Identity Confirmed
RNA Integrity	Capillary Gel Electrophoresis	s47 [redacted]	06-Jan-2022	s47 [redacted]
5'- Cap	RP-HPLC		07-Jan-2022	
Poly(A) Tail	ddPCR		12-Jan-2022	
Residual DNA Template	qPCR		07-Jan-2022	
Residual dsRNA	Immunoblot		11-Jan-2022	
Bacterial Endotoxin	Endotoxin (LAL)		02-Jan-2022	
Bioburden	Bioburden		09-Jan-2022	

Test missing - see below

Abbreviations: NTU = Nephelometric Turbidity Units; RT-PCR = Reverse Transcription PCR; dd-PCR = droplet digital PCR; IP-RP-HPLC = Ion-Pair Reversed-Phase HPLC; qPCR = quantitative PCR; dsRNA = double stranded RNA; LAL = Limulus Amebocyte Lysate; EU = Endotoxin Unit; CFU = Colony Forming Unit

Test Missing
Poly(A) Tail Length (spec - Poly(A) Tail Length Confirmed)

Added to DS release tests for Bivalent formulation in PM-2022-03551-1-2 and for the Monovalent formulation in PM-2021-04026-1-2

8.11.22 s22 [redacted]

All results comply

COMIRNATY Genealogy Flow

Process Stage	Batch Number	Input Process Stage	Input Batch Number(s)
Working Cell Bank	DW8970	N/A	N/A
Master Cell Bank Omicron	FT1817	N/A	N/A
Linearized DNA Template	FJ8256	Working Cell Bank	DW8970
Linearized DNA Template	FJ9482	Working Cell Bank	DW8970
Linearized DNA Template Omicron	FT0614	Master Cell Bank Omicron	FT1817
BNT162b2 Drug Substance	FR6462	Linearized DNA Template	FJ8256
BNT162b2 Drug Substance	FT3266	Linearized DNA Template	FJ8256, FJ9482
BNT162b2 Omicron Drug Substance	2241554_MB0011	Linearized DNA Template Omicron	FT0614
BNT162b2 Omicron Drug Substance	2241554_MB0016	Linearized DNA Template Omicron	FT0614
LNP Fabrication and Bulk Drug Product Formulation	GE5848	BNT162b2 Drug Substance & BNT162b2 Omicron Drug Substance	2241554_MB0011, 2241554_MB0016, FR6462, FT3266
Semi-Finished Goods	GE8400	LNP Fabrication and Bulk Drug Product Formulation	GE5848
Packaged Lot	GE8382	Semi-Finished Goods	GE8400

Prepared By:

s22 s22

22 Sep 2022 17:46:054-0400

REASON: I approve this document.

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Approved By:

s22 s22

23 Sep 2022 02:03:029-0400

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1 ✓

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Time Stamp	User	Action	Details
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22 Sep 2022 17:46:030-0400	s22 [redacted] UUID : 27b6bf54-6923-48a1-b9b7-97c9993c864f Email s22 [redacted]@pfizer.com	RequestSent	Sign request sent to ePak recipient.
22 Sep 2022 17:46:030-0400	s22 [redacted] UUID : d007747d-0c63-4417-9d1e-20158a2a4d4b Email s22 [redacted]@pfizer.com	RequestSent	Sign request sent to ePak recipient.
22 Sep 2022 17:46:032-0400	s22 [redacted] UUID : d007747d-0c63-4417-9d1e-20158a2a4d4b Email s22 [redacted]@pfizer.com IP Address : 168.224.160.14	DocumentViewed	Document viewed by signer.
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22 Sep 2022 17:46:054-0400	s22 [redacted] UUID : d007747d-0c63-4417-9d1e-20158a2a4d4b Email s22 [redacted]@pfizer.com IP Address : 168.224.160.14	Signed	The recipient signed the document with no comments. Comments: None Reason: I approve this document. I approve this document. I approve this document. Consent: I understand that my Electronic Signature is Equivalent to my Handwritten Signature and is therefore legally binding. My Electronic Signature will remain unique to me, and under no circumstance I am allowed to disclose my password to any individual which may allow unauthorized access to system. I understand that I am accountable and responsible for all actions associated with my Electronic Signature.
23 Sep 2022 02:02:058-0400	s22 [redacted] UUID : 27b6bf54-6923-48a1-b9b7-97c9993c864f Email s22 [redacted]@pfizer.com IP Address : 168.224.160.14	DocumentViewed	Document viewed by signer.
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23 Sep 2022 02:03:029-0400	s22 [redacted] UUID : 27b6bf54-6923-48a1-b9b7-97c9993c864f Email : s22 [redacted] pfizer.com IP Address : 168.224.160.14	Signed	The recipient signed the document with no comments. Comments: None Reason: I approve this document. I approve this document. I approve this document. Consent: I understand that my Electronic Signature is Equivalent to my Handwritten Signature and is therefore legally binding. My Electronic Signature will remain unique to me, and under no circumstance I am allowed to disclose my password to any individual which may allow unauthorized access to system. I understand that I am accountable and responsible for all actions associated with my Electronic Signature.
23 Sep 2022 02:03:029-0400	s22 [redacted] UUID : d007747d-0c63-4417-9d1e-20158a2a4d4b Email s22 [redacted] pfizer.com	Completed	The ePak is completed successfully.