Comparability Report for the Commercial Drug Substance Manufacturing Process of BNT162b2

Version 1.0

Date: see date of last signature

Test item: BNT162b2, PPQ batch comparison

Table ofContents

Table of Contents

List of Abbreviations

B	Brown
BNT	BioNTech
CFU	Colony forming unit
CFT	Controlled Freeze Thaw
CPPS	Critical process parameters
CRM	Clinical reference material
CTM	Clinical trial material
ddPCR	Droplet digital PCR
dsRNA	Double stranded RNA
EU	Endotoxin unit
EVA	Ethyl vinyl acetate
FFT	Flexible Freeze Thaw
HOS	Higher order structure
HTR	Historical test result
IM	Intramuscular
IPT-C	In-process test for control
IPT-M	In-process test for monitoring
LAL	Limulus amoebocyte lysate
LNPS	Lipid nanoparticles
MA	Marketing authorization
N/A	not applicable
NTU	Nephelometric Turbidity Units
PPQ	Process Performance Qualification
qPCR	Quantitative PCR
RBD	Receptor Binding Domain of the SARS-cov-2 virus
REN	Rentschler
RNA	Ribonucleic acid
RP-HPLC	Reverse phase high performance liquid chromatography
RT-PCR	Reverse transcription polymerase chain reaction
TFF	Tangential Flow Filtration
UFDF	Ultrafiltration/diafiltration
UTP	Uridine triphosphate

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Meaning of the signatures:

Person responsible for the study: I am responsible for the content of the study plan and confirm that the proposed study will be performed according to the SOPs and methods as well as the guidelines and regulations described in the plan.

Author: I am the author of this document.

Reviewer: I reviewed the study plan and confirm that this document is compliant with the scientific and technical standards and requirements.

Approved by (QA): I confirm that this document complies with the relevant quality assurance requirements.

1 General Information

1.1 Sponsor and Test Facilities

Sponsor

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2 Scope

The Marketing Authorisation Application of "Comirnaty - COVID-19 mRNA Vaccine (nucleoside modified)" EU/1/20/1528/001 describes two BNT162b2 drug substance (DS) manufacturing sites: 1) BioNTech in Mainz, Germany continued by Rentschler in Laupheim, Germany and 2) Pfizer in Andover, USA. In Europe, the DS production process is split into two part and involved two sites: BioNTech Manufacturing GmbH, An der Goldgrube 12, 55131 Mainz, Germany for process steps: In Vitro Transcription, DNase I Digestion and Proteinase K Digestion and Rentschler Biopharma SE, Erwin-Rentschler-Str. 21, 88471 Laupheim, Germany for Purification, Final Filtration & Dispense.

In order to increase production capacities in response to the demand in the global pandemic situation and in addition to ensure business continuity, an additional DS supply site will be set up. This process is executed by a tech transfer for BNT162b2 from the above mentioned sites to BioNTech Manufacturing Marburg GmbH, Emil-von-Behring-Straße 76, 35041 Marburg, Germany and will be supported by this interim comparability report.

2.1 Purpose

The purpose of this comparability report is to demonstrate comparability of the BNT162b2 DS manufacturing processes between the transferring and the receiving sites as stated in the underlying protocol. This summary includes data from all three PPQ batches manufactured at BNT Marburg supported by additional data from a technical batch performed prior to PPQ. As indicated in the comparability protocol, solely data from PPQ batches will be used for the final comparability statement. All elements in scope of the comparability protocol are addressed.

2.2 Introduction

BioNTech and Pfizer have been granted a marketing authorization (MA) by European Commission on December 2020 on a vaccine (Cormirnaty) to prevent Coronavirus Disease 2019 (COVID-19) caused by the virus SARS-CoV-2 for adults older than 16 years (Procedure No. EMEA/H/C/005735/0000, MA (EU) No.: EU/1/20/1528/001). The vaccine is based on full-length SARS-CoV-2 spike (S) glycoprotein antigen encoded in nucleoside-modified messenger RNA and formulated in lipid nanoparticles (LNPs), referred to as Comirnaty, COVID-19 mRNA Vaccine BNT162b2 or Pfizer-BioNTech COVID-19 vaccine or BNT162b2.

Several clinical studies are investigating BNT162b2 worldwide. The most relevant study for the registration is the Phase 1/2/3 study (named BNT162-02 (BioNTech code) / C4591001 (Pfizer code), which is currently performed in the US (start 4 May 2020; NCT04368728), Argentina, Brazil, Turkey, Germany, and South Africa.

The Phase 2/3 was enrolled up to 43,998 subjects (randomized 1:1 to BNT162b2 or matching placebo) for an adequately powered efficacy assessment in addition to safety and exploratory immunogenicity assessments.

The development of the commercial DS manufacturing process includes linearized plasmid DNA as a template and uses tangential flow filtration (TFF) for RNA purification. This process was established in parallel at two sites i) BioNTech, Mainz, Germany (incl. Rentschler, Laupheim, Germany for RNA purification) and ii) Pfizer, Andover, USA.

For production capacity increase and ensurance, an additional DS manufacturing site is planned to be

registered for commercial supply of Comirnaty. The DS manufacturing process was transferred from BioNTech Mainz / Rentschler Laupheim to BioNTech Manufacturing Marburg GmbH, Emil-von-Behring-Straße 76, 35041 Marburg, Germany.

The comparability report described herein, focusses on an assessment of commercial BNT162b2 DS manufacturing processes as well as the analytical testing between BioNTech Mainz / Rentschler Laupheim and BioNTech Marburg. The report shall clearly demonstrate the comparability of the newly introduced manufacturing processes for all steps and unit operations performed in Marburg with the sending sites and should allow for the future comparison and alignment of testing and processes. All elements in scope of the comparability protocol are addressed and comparability statements are documented by validated data and statistical assessments.

2.3 Product Outline

The nomenclature of the BNT162b2 Drug Substance is given in **Table 1**.

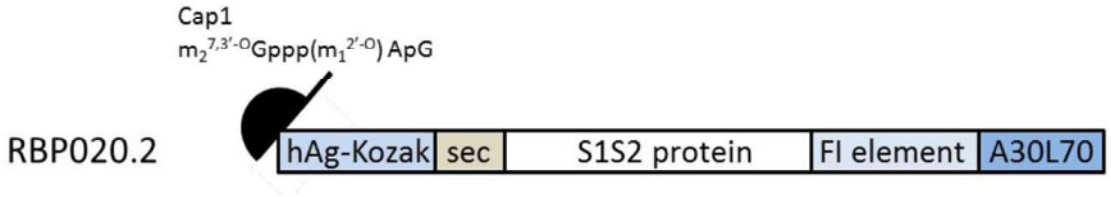
Table 1*:* Nomenclature of BNT162b2 Drug Substance.

Product code:	BNT162b2
Chemical class:	Ribonucleic Acid (RNA)
Encoded antigen:	Viral spike protein (S1S2 protein) of the SARS-CoV-2 (S1S2 full-length protein, containing two mutations: K986P and V987P)
CAS Registry Number:	2417899-77-3
CA Index Name:	[redacted]

4.2 1st ind.

BNT162b2 drug substance is a single-stranded, 5'-capped mRNA that is translated into a protein (the encoded antigen). Figure 1 schematizes the general structure of the antigen-encoding RNA, which is determined by the respective nucleotide sequence of the DNA template used as template for in vitro RNA transcription. In addition to the codon-optimized sequence encoding the antigen, the RNA contains common structural elements optimized for mediating high RNA stability and translational efficiency (5'-cap, 5'-UTR, 3'-UTR, poly(A)-tail; see below). Furthermore, an intrinsic signal peptide (sec) is part of the antigen-encoding regions and is translated as an N-terminal tag. The RNA does not contain any Uridines: instead of Uridine the modified analog N1-methylpseudouridine is used in RNA synthesis.

Figure 1: Schematic illustration of the general structure of the BNT162b2 drug substance with 5'-cap, 5'- and 3'-untranslated regions (hAg-Kozak and FI element, respectively), coding sequence with mutations and intrinsic signal peptide (sec) as well as poly(A)-tail (A30L70). Individual elements are not drawn to scale compared to their respective sequence lengths.



3 Description of the planned change (Site transfer)

3.1 Marburg Process and Facility Description

The BNT162b2 DS manufacturing commercial process was transferred from BioNTech Mainz / Rentschler Laupheim to Marburg. The BNT162b2 DS manufacturing process consists of five steps:

- Step 1: In Vitro Transcription (mRNA synthesis)
- Step 2: DNase I Digestion
- Step 3: Proteinase K Digestion
- Optional pool transportation to other sites for purification Step 4.
- Step 4: Purification
- Step 5: Final Filtration and Dispense

4 Drug Substance Comparability Assessment

The following table provides an overview on the individual process steps and unit operations performed for drug substance manufacturing at the three sites in scope of this assessment.

Drug substance manufacturing of BNT162b2 at the receiving site BNT Marburg includes all unit operations shown below, whereas the sending site BNT Mainz performs manufacturing (IVT) and Rentschler performs purification as well as final filtration & dispense. The respective unit operations in Marburg will be compared to the sending site applicable.

Table 2: Comparability assessment outline for DS manufacturing: IVT and RNA generation

Site		BioNTech Manufacturing GmbH, Mainz, Germany	BioNTech Manufacturing Marburg GmbH
Batch Number for comparability exercise		20E162001 20E162002 20E162003	Technical Batch MBT004 (supporting data) PPQ1 (MB0001) PPQ2 (MB0002) PPQ3 (MB0003)
In Vitro Transcription	Scale	4.2 1st ind.	4.2 1st ind.
	DNA template	Linearized plasmid DNA	Linearized plasmid DNA
DNase I Digestion		DNase I digestion	DNase I digestion
Proteinase K Digestion		Proteinase K digestion	Proteinase K digestion
Storage IVT material		4.2 1st ind.	4.2 1st ind.

Table 3: Comparability assessment outline for DS manufacturing: Purification and Final Filtration & Dispense

Site	**Rentschler Biopharma SE**, Laupheim, Germany	**BioNTech Manufacturing Marburg GmbH**
Batch Number for comparability exercise	1071539 1071542 1071544	Tech Batch MBT004 (supporting data) PPQ1 (MB0001) PPQ2 (MB0002) PPQ3 (MB0003)
Purification Method	Tangential Forward Flow	Tangential Forward Flow
Filtration & Dispense	Final Filtration & Dispense After filling in [redacted] bags storage at [redacted]	Final Filtration & Dispense After filling in [redacted] bags hold at [redacted]

4.1 Results of the Comparability Assessment

The comparability exercise consists of the following topics and results are presented in the sections below.

- Batch selection (section 4.2)
- Location of manufacturing steps (zoning concept) (section 4.3)
- Equipment used for DS manufacturing (section 4.4)
- Raw materials and buffers (section 4.5)
- Comparability of process parameters (section 4.6)
- Packaging materials DS bulk (section 0)
- Composition of the drug substance (section 4.9)
- DS release specifications (section 4.10)
- DS stability testing (section 4.11)
- Extended characterization data Drug Substance (section 4.12)

4.2 Batch Selection

In this exercise, the comparability of the commercial production and testing methods of the aforementioned sites should be demonstrated. In doing so, the process performance qualification batches (PPQ) of the involved sites will be compared and form part of the final comparability statement. The results from the Technical Batch MBT004 produced at BNT Marburg will be further used to support the assessment by additional data. Batches from BNT Mainz / Rentschler have already been produced and data is available. Marburg batches in scope of this report were manufactured as stated in **Table 4**.

Table 4: List of batches used for drug substance comparability.

Manufacturing Site	Drug substance Batch(es)	Process	Batch scale (L)	Date of Manufacture	Purpose of Material
BioNTech Manufacturing GmbH, Mainz / Rentschler Laupheim	20E162001 / 1071539	BNT / REN	4.2 1st ind.	25-SEP-2020	PPQ 1, Stability
	20E162002 / 1071542	BNT / REN		01-OCT-2020	PPQ 2, Stability
	20E162003 / 1071544	BNT / REN		07-OCT-2020	PPQ 3, Stability
BioNTech Manufacturing Marburg GmbH, Marburg	Tech Batch (MBT004)	MR		12-FEB-2021	Technical Batch (supporting data)
	PPQ1 (MB0001)	MR		20-FEB-2021	PPQ 1 Batch, Stability
	PPQ2 (MB0002)*	MR		23-FEB-2021	PPQ 2 Batch, Stability
	PPQ3 (MB0003)	MR		28-FEB-2021	PPQ 3 Batch, Stability

4.2 1st ind.

4.3 Location of manufacturing steps (zoning concept)

4.2 1st ind.

4.4 Equipment used for the individual manufacturing steps

A comparison of the equipment used during manufacturing of BNT162b2 drug substance at the transferring sites (TS) and the receiving site (RS) can be found in the following tables. At all sites, the equipment used for cGMP manufacture is/will be qualified for its intended use.

A tabulated comparison of the equipment used in the different unit operations is given in **Table 5**. Equipment that is not equal, identically constructed or from different vendors was identified in a gap assessment and can be found in **Table 6**. The latter equipment was assessed on possible impacts on production processes or product quality (h: high, m: medium, l: low). Furthermore, a mitigation strategy was established in order to assure comparability in terms of output parameters and product quality control. Consequently, comparability can be claimed for this element.

Table 5: Summary of comparison of equipment used in different facilities.

Process step	Manufacturing Site		
	BNT Mainz / Rentschler	BNT Marburg	Comment
In Vitro Transcription	[redacted]		
DNase I Digestion			
Proteinase K Digestion			
Storage of IVT			
TFF			
Final Filtration			
Final Storage			

Table 6: Details and risk mitigation strategy regarding production equipment.

Differences between BioNTech Mainz / Rentschler and BioNTech Marburg	Ranking (H/M/L)	Risk mitigation strategy
4.2 1st ind.		

4.5 Comparison of raw materials and buffer

Raw materials and consumables used for the IVT process are comparable between the sending and receiving site as can be seen from **Table 7**. A gap assessment did not show any significant differences in terms of materials used such as enzymes, buffers and NTPs due to ordering of equal ready-to-use materials from either the same vendors or of the same quality.

4.2 1st ind.

Consequently, comparability can be claimed for buffers and raw materials.

Table 7: Raw Material comparison between sending and receiving sites (source: BNT Marburg Material-GAP Assessment and DS GAP Assessment).

Raw Material	Manufacturing Site		
	BNT Mainz / Rentschler	BNT Marburg	Comment
4.2 1st ind.			

4.6 Manufacturing Processes and Controls – Scope and Approach of the Comparability Assessment

This comparability exercise challenged the manufacturing of BNT162b2 drug substance produced by the receiving site BioNTech Manufacturing Marburg GmbH, Marburg with the transferring sites BioNTech Manufacturing GmbH, Mainz / Rentschler, Laupheim for commercial processes.

Table 4 shows the batches used for this comparability study. The exercise includes all data from the three PPQ batches produced at BNT Marburg as well as of one technical batch manufactured prior to PPQ to support the study.

4.2 1st ind. [redacted]

4.7 Comparability criteria for process parameters

In general, comparability is demonstrated in case the parameters meet their pre-defined acceptance ranges shown in the tables below. 4.2 1st ind. [redacted]

Table 8: Differences identified between BNT Mainz / Rentschler and Marburg in a gap assessment.

Differences between BioNTech Mainz / Rentschler and BioNTech Marburg	Ranking (H/M/L)	Risk mitigation strategy
4.2 1st ind. [redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

For the drug substance manufacturing process, critical process parameters (CPPs) have been defined (**Table 9** to **Table 12**) and in-process tests for monitoring (IPT-M) and for control (IPT-C) are established to ensure consistent manufacturing (**Table 13**).4.2 1st ind.

For the comparability assessment of the DS manufacturing processes, CPP and IPT values obtained during manufacturing of each batch in scope of this exercise will be compared to the acceptable range defined for each critical parameter within each manufacturing process step.4.2 1st ind.

The following tables show the process control for each manufacturing unit operation, comparing parameters, controls and acceptable ranges.4.2 1st ind.

4.2 1st ind.

Table 9: Process Controls In Vitro Transcription.

Process Parameter Controls: In Vitro Transcription							
Parameter	**Category**	**Acceptable Range/Limit**	**Tech Batch MBT004 (supporting data)**	**PPQ1 Batch**	**PPQ2 Batch**	**PPQ3 Batch**	**Comparability Statement**
[redacted]							Comparability confirmed
							Comparability confirmed
							Comparability confirmed
							Comparability confirmed
							Comparability confirmed
							Comparability confirmed
							Comparability confirmed
							Comparability confirmed
							Comparability confirmed
							Comparability confirmed
							Comparability confirmed

Table 10: Process Controls DNase I Digestion.

Process Parameter Controls: DNase I Digestion							
Parameter	Category	Acceptable Range/Limit	Tech Batch MBT004 (supporting data)	PPQ1 Batch	PPQ2 Batch	PPQ3 Batch	Comparability Statement
[redacted]							Comparability confirmed
							Comparability confirmed
							Comparability confirmed
							Comparability confirmed

Table 11: Process Controls Proteinase K Digestion.

Process Parameter Controls: Proteinase K Digestion							
Parameter	Category	Acceptable Range/Limit	Tech Batch MBT004 (supporting data)	PPQ1 Batch	PPQ2 Batch	PPQ3 Batch	Comparability Statement
[redacted]							Comparability confirmed
							Comparability confirmed
							Comparability confirmed

Table 12: Process Controls Tangential Flow Filtration and Formulation.

Process Parameter Controls: Tangential Flow Filtration and Formulation							
Parameter	**Category**	**Acceptable Range/Limit**	**Tech Batch MBT004 (supporting data)**	**PPQ1 Batch**	**PPQ2 Batch**	**PPQ3 Batch**	**Comparability Statement**
[redacted]							**Comparability confirmed**
							Comparability confirmed
							Comparability confirmed

4.2 1st ind.

Table 13: In-process testing (IPT-C and IPT-M) during DS manufacturing.

In-Process Testing (IPT-M and IPT-C) during DS Manufacturing											
Production Step	**Sample definition**	**Quality Attribute**	**Analytical Procedure**	**Category**	**Acceptable Range/Limit**	**Criteria for Comparability****	**Tech Batch MBT004 (supporting data)**	**PPQ1 Batch**	**PPQ2 Batch**	**PPQ3 Batch**	**Comparability Statement**
[redacted]											**Comparability confirmed**
											Comparability confirmed
											Comparability confirmed
											Comparability confirmed
											Comparability confirmed
											Comparability confirmed

4.2 1st ind.

In-Process Testing (IPT-M and IPT-C) during DS Manufacturing											
Production Step	**Sample definition**	**Quality Attribute**	**Analytical Procedure**	**Category**	**Acceptable Range/Limit**	**Criteria for Comparability****	**Tech Batch MBT004 (supporting data)**	**PPQ1 Batch**	**PPQ2 Batch**	**PPQ3 Batch**	**Comparability Statement**
4.2 1st ind.											

4.2 1st ind. Both values are available and will be subject to this comparability exercise.

** Comparability and acceptance of the results will also consider trends that might be observed from the PPQ batches. Trends will require further investigation and explanation.

‡ A comparison against historic batches 4.2 1st ind. will be applied. A statistical approach was performed for 8 historic batches (**Table 17:** 20E162004 to 20E162011). 4.2 1st ind.

♣ The RNA content is 4.2 1st ind. . A statistical approach is not applicable.

ª Hold time was 4.2 1st ind.

Abbreviations: IPT-M: In-Process-Test for monitoring, IPT-C: In-Process-Test for control.

4.2 1st ind.

Table 14: Process Performance Monitoring – Step Yields

Process Yields						
Process Step	**Acceptable Range/Limit**	**Tech Batch MBT004 (supporting data)**	**PPQ1 Batch**	**PPQ2 Batch**	**PPQ3 Batch**	**Comparability Statement**
[redacted]						**Comparability confirmed**
[redacted]						**Comparability confirmed**
[redacted]						**Comparability confirmed**

4.2 1st ind.

4.8 Packaging Materials for DS Bulk Material

BNT162b2 drug substance is filled and stored in 4.2 1st ind. containers. 4.2 1st ind. containers are sterile, single-use, flexible, disposable bags specifically designed for freezing, thawing and frozen storage of biopharmaceuticals. The container closure system is equivalent and the product contact layer is identical for both 4.2 1st ind. containers used at the transferring and receiving site.

Table 15: Comparison of containers used for drug substance

	BNT/REN	BNT Marburg
DS Container	4.2 1st ind.	4.2 1st ind.

4.9 Composition

The composition of BNT162b2 drug substance is comparable at both sites in terms of the formulation buffer used (10 M HEPES, 0.1 mM EDTA, pH 7.0), pH (7.0 ± 0.5) and final RNA concentration (2.25 ± 0.25 mg/mL). As can be seen from **Table 16**, drug substance composition is comparable at transferring and receiving site. Raw materials and buffers used have been addressed in **Section 4.5**.

Table 16: Drug substance composition

Attribute	BNT/REN	BNT Marburg
Formulation buffer	10 mM HEPES, 0.1 mM EDTA, pH 7.0	
pH	7.0 ± 0.5	
Content (RNA Concentration)	2.25 ± 0.25 mg/mL	

4.10 Drug Substance Release Specifications

The analytical testing strategy applied to BNT162b2 has evolved during the development history for the product. In preparing the analytical testing panel for setting of acceptance criteria, improved methods for the assessment of product quality have been introduced late in development. Quality attributes (QAs) of the BNT162b2 drug substance were identified and assessed for criticality. DS attributes were initially identified based on their potential relationship to product quality.

Table 18 summarizes DS control attributes designated as quality attribute (QA) or critical quality attribute (CQA) according to submission module 3.2.S.2.6. DS release specifications are equal at transferring and receiving sites and therefore, are suitable for the comparability exercise. 4.2 1st ind.

[redacted]

4.2 1st ind.

[redacted]

Table 17: Historic batches used for the statistical assessment and comparability against BNT Marburg PPQ batches.

IVT date	DNA Lot	BNT Mainz IVT Lot	Rentschler Lot
22.09.2020	CPF-L002	20E162001	1071539
29.09.2020	CPF-L002	20E162002	1071542
02.10.2020	CPF-L001	20E162003	1071544
12.10.2020	CPF-L002	20E162004	1071545
14.10.2020	CPF-L003	20E162005	1071546
19.10.2020	CPF-L003	20E162006	1071547
21.10.2020	CPF-L004	20E162007	1071548
02.11.2020	CPF-L004	20E162008	1071551
04.11.2020	CPF-L004	20E162009	1071552
09.11.2020	CPF-L004	20E162010	1071553
11.11.2020	CPF-L004	20E162011	1071554

Table 18: Drug substance release specifications.

Sample definition	Quality Attribute	Analytical Procedure	Acceptance Criteria		Mean‡	Standard Deviation‡	3 Sigma Range‡	Min / Max	Test Location	Criteria for Comparability*	Tech Batch MBT004 (supporting data)	PPQ1 Batch	PPQ2 Batch	PPQ3 Batch	Comparability Statement
			BNT/REN	BNT Marburg											
DS-REL-Clarity	Clarity[a]	Appearance (visual inspection)[a]	4.2 1st ind.		N/A	N/A	N/A	N/A	4.2 1st ind.	Complies defaults	4.2				Comparability confirmed
DS-REL-Color	Colorstion[d]	Appearance (visual inspection)[a]	Not more intensely colored than level [redacted] of the brown (B) color standard		N/A	N/A	N/A	N/A		Complies defaults	complies	complies	complies	complies	Comparability confirmed
DS-REL-pH	pH	Potentiometric[a]	4.2 1st ind.		4.2 1st ind.	4.2 1st ind.				Complies defaults and limit fulfilled			4.2.1		Comparability confirmed
DS-REL-RNACont	Content (RNA concentration)	UV Spectroscopy								Limits fulfilled	4.2.1st				Comparability confirmed
DS-REL-Identity	Identity of encoded RNA sequence	RT-PCR	Identity confirmed		N/A	N/A	N/A	N/A		Complies defaults	confirmed	confirmed	confirmed	confirmed	Comparability confirmed
DS-REL-Integrity	RNA integrity	Capillary gel electrophoresis	4.2 1st ind.		4.2 1st ind	4.2 1st ind				Complies defaults and limit fulfilled				4.2	Comparability confirmed
DS-REL-5cap	5'-Cap[b]	RP-HPLC				4.2 1st ind				Complies defaults and limit fulfilled	4.2				Comparability confirmed
DS-REL-PolyA	Poly(A) tail	ddPCR				4.2 1st ind				Complies defaults and limit fulfilled	4.2				Comparability confirmed

Sample definition	Quality Attribute	Analytical Procedure	Acceptance Criteria		Mean‡	Standard Deviation‡	3 Sigma Range‡	Min / Max	Test Location	Criteria for Comparability*	Tech Batch MBT004	PPQ1 Batch	PPQ2 Batch	PPQ3 Batch	Comparability Statement
			BNT/REN	BNT Marburg											
DS-REL-resiDNA	Residual DNA template	qPCR	4.2 1st ind.				4.2 1st ind.		4.2 1st ind.	Complies defaults and limit fulfilled	4.2				Comparability confirmed
DS-REL-dsRNA	double stranded RNA (dsRNA)	Immuno-based assay	4.2 1st ind.		N/A	N/A	N/A	N/A		Complies defaults[A]	4.2 1st				Comparability confirmed
DS-REL-Endo	Bacterial endotoxins	Endotoxin (LAL) [a]	4.2 1st ind.		N/A	N/A	N/A	N/A		Complies defaults[A]	4.2 1st				Comparability confirmed
DS-REL-Bio	Bioburden	Bioburden (TAMC und TYMC) [a]	4.2 1st ind.		4	N/A	N/A	N/A		Complies defaults[A]	4				Comparability confirmed

* In case 4.2 1st ind. values do not meet the historic 3-sigma range from 4.2 1st ind. a comprehensive investigation will be triggered. The outcome of the investigation needs to be considered to assess whether comparability is affected. The filed acceptance criteria are mandatory to judge product quality.

‡ Value or range calculated from historic batches (Table 17).

A Analytical method or values obtained do not allow for a statistical assessment due to values below limit of quantification or quantitative method.

1 4.2 1st ind.

a. Compendial

b. The basis for the establishment of the historical range was 11 batches tested in three independent sequences, which is a limited database. Consequently, we included another 14 batches (all batches produced so far) from 4.2 1st ind. to establish a more representative database and recalculated adjusted 3-sigma ranges on this extended database. New ranges are as following: 3-sigma: 4.2 1st ind.

c. At the test is carried out as limit test with comparison to the standard for 4.2 1st ind. Therefore, the outcome is either less than equal than or above

d. The color test is a limit test and therefore the result is reported as complies when the results is not more than 4.2 on the Ph. Eur. color scale

Abbreviations: N/A: not applicale; NTU: Nephelometric Turbidity Units; B: brown; RT-PCR: reverse transcription polymerase reaction; ddPCR: droplet digital PCR; qPCR: quantitative PCR; dsRNA: double stranded RNA; LAL: Limulus amebocyte
lysate: endotoxin unit; CFU: colony forming unit.

Graphical evaluation of comparability has been conducted for numerical parameters. Descriptive parameters or parameters, which are reported as greater than (>) or less than (<) are not shown in the graphic section as well but are listed in **Table 18**.

Figure 2 shows the individual values chart (I Chart) for the pH value of the technical batch (shown as triangle) and three PPQ batches (shown as dots). 4.2 1st ind. . However, comparability is confirmed due to results, which meet the acceptance criteria without any unexpected trends observed.

Figure 3 shows the I Chart for the Content (RNA concentration) in mg/mL for the technical batch (triangle) and three PPQ batches (dots). 4.2 1st ind.

4.2 1st ind.

Thus, comparability is confirmed.

Figure 4: I Chart of the RNA Integrity in percent for the technical run (triangle) and three PPQ batches (dots)

Figure 4 shows the I Chart for the RNA Integrity in percent for the technical batch (triangle) and three PPQ batches (dots). 4.2 1st ind.

Thus, comparability is confirmed.

Figure 5 shows the I Chart for the 5'cap in percent for the technical batch (triangle) and three PPQ batches (dots). 4.2 1st ind.

4.2 1st ind.

Thus, comparability is confirmed.

Figure 6 shows the I Chart for the Poly(A) Tail in percent for the technical batch (triangle) and three PPQ batches (dots). 4.2 1st ind.

Thus, comparability is confirmed.

Figure 7 shows the I Chart for the residual DNA Template in percent for the technical batch (triangle) and three PPQ batches (dots). 4.2 1st ind.

4.2 1st ind.

Thus, comparability is confirmed.

Figure 8 shows the I Chart for the double stranded RNA in pg DS/µg RNA for for the technical batch (triangle) and three PPQ batches (dots). 4.2 1st ind.

Thus, comparability is confirmed.

In summary, all drug substance release parameters are well within the specification limits and meet the predefined historical limits calculated from BNT Mainz/Rentschler HTRs. It can be concluded that no adverse or unexpected trends were observed and all attributes meet their acceptance criteria. Therefore, it can be concluded that the manufacturing process in Marburg is comparable to the transferring site and furthermore, is capable to generate a product of comparable quality.

4.11 Drug Substance Stability Testing

Drug substance material from all three BNT Marburg PPQ batches was used for stability testing as can be seen from **Table 19**. An interim report (STAB_STRE_00597004) was generated to summarize all stability results available at intended conditions -20 °C ± 5 °C, accelerated conditions 5 °C ± 3 °C and stressed conditions 25 °C ± 2 °C / 60% ± 5% RH. The pull points and acceptance criteria were defined according to stability protocol STAB_STPR_00547471.

Table 19: BNT162b2_DS_001-009 batches pull points dates

Stability Study No	Batch No.	Pull point: week [W] / Month [M]	Dates
BNT162b2_DS_001 BNT162b2_DS_002 BNT162b2_DS_003	MB0001	4.2 1st ind.	
BNT162b2_DS_004 BNT162b2_DS_005 BNT162b2_DS_006	MB0002		
BNT162b2_DS_007 BNT162b2_DS_008 BNT162b2_DS_009	MB0003		

#1 As per SOP-8005644, the storage date defines the study start (T0)

The following test methods prescribed in the shelf life specifications of Control Procedure [CP_AMS_00543201 (which is based in general on [SPEC-257-v03, closing Sequenz MAA] will be applied during this stability study.

Table 20 states all test parameters and the corresponding acceptance criteria according to [CP_AMS_00543201] applied in the stability study. The acceptance criteria are valid for the intended storage condition at -20 °C ± 5 °C. 4.2 1st ind.

Table 20: Methods used for stability studies and acceptance criteria

Analytical Method	Acceptance Criteria#1
Composition and Strength	
Appearance (Clarity) Ph. Eur. 2.2.1	4.2 1st ind.
Appearance (Coloration) Ph. Eur. 2.2.2	Not more intensely colored than level [redacted] of the brown (B) color standard
Content (RNA concentration) UV spectroscopy (A260nm)	4.2 1st ind.
pH Ph. Eur. 2.2.3/ USP <791>	4.2 1st ind.
Product purity	
RNA Integrity (Capillary Gel Electrophoresis)	4.2 1st ind. intact RNA
5'Cap (RP HPLC)	4.2 1st ind.
Poly(A) Tail	
Microbiological control	
Endotoxins (LAL) Ph. Eur. 2.6.14/ USP <791>	4.2 1st ind.
Bioburden/ Microbial Enumeration Test (MET) Ph. Eur. 2.6.12/ USP <61>	

In summary, all results of the tested batches MB0001, MB0002 and MB0003 up to today meet the predetermined acceptance criteria under intended, accelerated and stressed conditions. In addition, the results are within the expected analytical variability and no atypical results or trends were observed. Consequently, comparability can be stated with respect to the stability data already available. For further information regarding the stability study, please refer to the interim stability report mentioned above.

4.12 Extended Characterization Data Drug Substance

Extended characterization according to **Table 21** was performed for all BNT Marburg PPQ batches and were tested at Pfizer Analytical Research and Development (ARD). The technical batch MBT004 has not been part of the heightened characterization study due to limited testing capacities at the testing site as well as the non-commercial status of the latter batch.

Testing was performed side-by-side for the batches mentioned in **Table 22** and a comprehensive report (Pfizer Doc. No. INX100451011) was prepared, which includes all data and conclusions. For detailed information, please refer to report INX100451011, which is attached to this document.

In summary, all criteria for comparability according to **Table 21** were met and comparability between the transferring and the receiving site can be stated without restrictions.

Table 21: Additional Characterization for Drug Substance

Quality Attribute	Analytical Procedure	Type of Comparison	Acceptance Criteria	Comparability Statement
Purity				
5'- Cap (species ID)	RNAse H followed by LC-UV-MS	Side-by-side	4.2 1st ind. [redacted]	Comparability confirmed
Poly(A) Tail: Length and Distribution	Enzyme digestion (RNase T1+RNase A) followed by RPHPLC-UV	Side-by-side	4.2 1st ind. [redacted]	Comparability confirmed
Identity				
Expressed protein Western blot	Western blot	Side-by-side	4.2 1st ind. [redacted]	Comparability confirmed
RNA HOS	CD Spectra	Side-by-side		Comparability confirmed
Primary Structure	RNA Digest mapping	Side-by-side		Comparability confirmed

Table 22: Batches used for the extended characterization study

Manufacturing Site	Drug Substance Batch(es)	Process	Batch Scale (L)	Date of Manufacturing	Purpose of Material
Pfizer, Andover ACMF	20Y513C201	DS Process 2	4.2 1st ind. [redacted]	13-AUG-2020	Emergency supply, Stability
BioNTech Manufacturing GmbH, Mainz / Rentschler Laupheim	20E162001 / 1071539			24-SEP-2020	PPQ 1, Stability
	20E162002 / 1071542			30-SEP-2020	PPQ 2, Stability
	20E162003 / 1071544			06-OCT-2020	PPQ 3, Stability
BioNTech Manufacturing Marburg GmbH, Marburg	MB0001			20-FEB-2021	PPQ 1, Stability
	MB0002			23-FEB-2021	PPQ 2, Stability
	MB0003			28-FEB-2021	PPQ 3, Stability

5 Deviations and Observations

The following section provides an overview regarding the process deviations observed during manufacturing of the three PPQ batches and the technical batch at BNT Marburg. All deviation were comprehensively investigated with respect to their impact on the process and product quality attributes. Furthermore, an examination on the comparability exercise was performed for each deviation as agreed in the underlying comparability protocol. All deviations affecting comparability were completed and closed prior to generating this report.

In summary, no deviation was identified, which has a potential impact on the aforementioned attributes or this comparability exercise. Consequently, comparability can be stated without any restrictions.

Table 23: List of deviations occurred during the technical batch (MBT004) and the three PPQ batches manufactured at BNT Marburg.

Deviation ID	Title of Deviation	Status	Date of Occurrence	Classification
[redacted]				

6 Conclusion

With respect to all results generated for the three executed PPQ batches in terms of critical process parameters, in-process tests for monitoring/control, release tests, stability data, extended characterization results and deviations occurred, comparability between the transferring and the receiving site can be stated without any restrictions.

This conclusion results from meeting all acceptance criteria and historical limits 4.2 1st ind. [redacted] Moreover, a side-by-side comparison demonstrated comparable results for batches manufactured at BNT Mainz/Rentschler and BNT Marburg.

Deviation occurred during PPQ batch manufacturing were classified and comprehensively evaluated. No deviation was identified, which could have an impact on either validation activities or the comparability exercise.

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