

2.3.S.1. GENERAL INFORMATION

2.3.S.1.1. Nomenclature

Information on the nomenclature of BNT162b2 is provided in Table 2.3.S.1-1

Table 2.3.S.1-1. Nomenclature of BNT162b2 Drug Substance

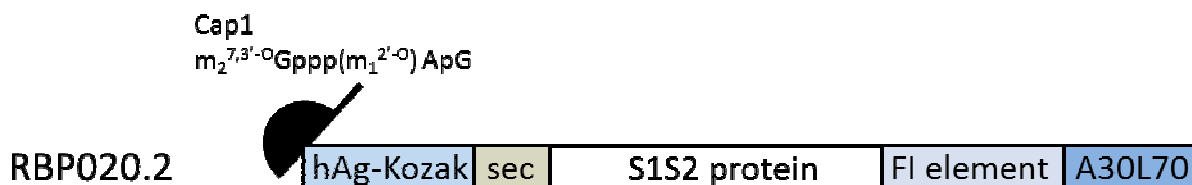
Product code:	BNT162b2
Laboratory code:	CCI
Chemical class:	Ribonucleic Acid (RNA)
Encoded antigen:	Viral spike protein (S1S2 protein) of the SARS-CoV-2 (S1S2 full-length protein, containing two mutations: K986P and V978P)
CAS Registry Number:	2417899-77-3
CA Index Name:	CCI
INN	Tozinameran (proposed INN)

2.3.S.1.2. Structure

BNT162b2 drug substance is a single-stranded, 5'-capped mRNA that is translated into a protein (the encoded antigen).

Figure 2.3.S.1-1 illustrates the general structure of the antigen-encoding RNA, which is determined by the respective nucleotide sequence of the DNA 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 open reading frame and is translated as an N-terminal peptide. The RNA does not contain any uridines; instead of uridine the modified N1-methylpseudouridine is used in RNA synthesis.

Figure 2.3.S.1-1. General structure of the RNA.

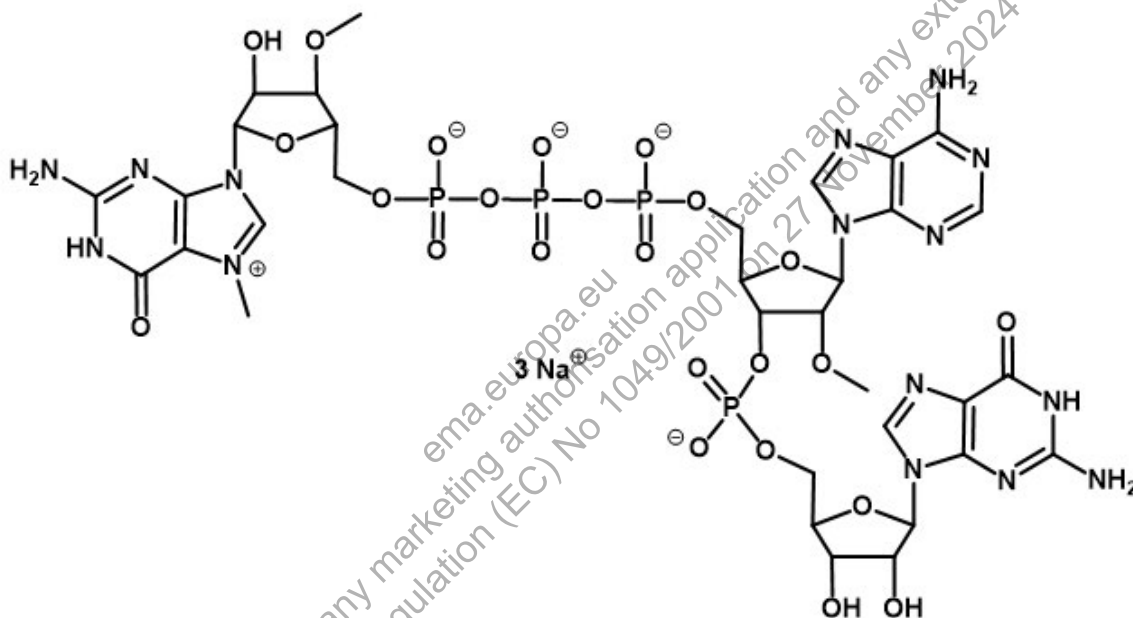


Schematic illustration of the general structure of the BNT162b2 drug substance with 5'-cap, 5'- and 3'-untranslated regions (5'UTR and 3'UTR), coding sequence with mutations and intrinsic signal peptide (sec) as well as poly(A)-tail. Individual elements are not drawn to scale compared to their respective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

mRNA cap

A cap1 structure $m_2^{7,3'-O}Gppp(m_1^{2'-O})ApG$ Figure 2.3.S.1-2 is utilized as specific capping structure at the 5'-end of the RNA drug substance (Figure 2.3.S.1-2).

Figure 2.3.S.1-2. 5'-cap analog ($m_2^{7,3'-O}Gppp(m_1^{2'-O})ApG$) for production of RNA containing a cap1 structure



The cap1 structure (i.e., containing a 2'-O-methyl group on the penultimate nucleoside of the 5'-end of the RNA chain) is incorporated into the BNT162b2 drug substance by using a respective cap analog during *in vitro* transcription. For RNAs with modified uridine nucleotides, the cap1 structure is superior to other cap structures, since cap1 is not recognized by cellular factors such as IFIT1 and, thus, cap1-dependent translation is not inhibited by competition with eukaryotic translation initiation factor 4E. In the context of IFIT1 expression, mRNAs with a cap1 structure give higher protein expression.

Further information is provided in [Section 3.2.S.1.2 Structure](#).

2.3.S.1.3. General Properties

The general properties of BNT162b2 drug substance formulated at a target concentration of 2.25 mg/mL in DS formulation buffer (CCI HEPES, CCI EDTA, CCI) are summarized in Table 2.3.S.1-2. The detailed descriptions of structural and functional studies conducted to characterize BNT162b2 are presented in [Section 3.2.S.3.1 Elucidation of Structure and Other Characteristics](#).

Table 2.3.S.1-2. BNT162b2 Drug Substance General Properties

Appearance	Clear to slightly opalescent, colorless to slightly brown liquid
Specific Absorption Coefficient (260 nm)	25 L/g × cm
Theoretical length^a	4,283 nucleotides
Theoretical mass^b	1,388,651 g/mol
pH	Target 7.0

a. Theoretical value has been verified by gel electrophoresis compared to a size marker. The length is 4,284 nucleotides when the presence of the 5'-cap analog (G) is included.

b. Theoretical value has been verified indirectly by control of RNA lengths.

2.3.S.2. MANUFACTURE

2.3.S.2.1. Manufacturer(s)

Table 2.3.S.2-1 lists the sites that have responsibilities in the production of RNA drug substance and their specified functions.

Table 2.3.S.2-1. Sites and Responsibilities for Manufacture and Testing of BNT162b2 Drug Substance

Site	Responsibility
Wyeth BioPharma Division of Wyeth Pharmaceuticals, LLC ^a 1 Burtt Road Andover, MA 01810 United States	Manufacture of drug substance Release and Stability Testing (Composition, Strength, Identity, Purity, Process Related Impurities, Safety)
Pfizer Inc 875 Chesterfield Parkway West Chesterfield, MO 63017 United States	Release and Stability Testing (Composition, Strength, Identity, Purity, Process Related Impurities)
BioNTech Manufacturing GmbH An der Goldgrube 12 55131 Mainz Germany	Manufacture of drug substance (In-vitro Transcription, DNase I and Proteinase K digestion) Release and Stability Testing (Identity, Purity, Process Related Impurities)
Rentschler Biopharma SE Erwin-Rentschler-Str. 21 88471 Laupheim Germany	Manufacture of drug substance (Ultrafiltration/Diafiltration (UFDF), DS Filling) Release and Stability Testing (Composition, Strength, Safety)
BioNTech Innovative Manufacturing Services GmbH Vollmersbachstraße 66 55743 Idar-Oberstein Germany	Release and Stability Testing (Product Related Impurities, Purity)

a. The legal entity name change from Wyeth BioPharma Division of Wyeth Pharmaceuticals was changed at the acquisition by Pfizer in 2009, since then the Wyeth Pharmaceuticals manufacturing site in Andover, Massachusetts belongs to Pfizer's production sites and is embedded in Pfizer's GMP system. Pfizer will be utilized throughout the CTD.

2.3.S.2.2. Description of Manufacturing Process and Process Controls

Data for this section is pending and will be updated once the data has been generated, analyzed, and verified.

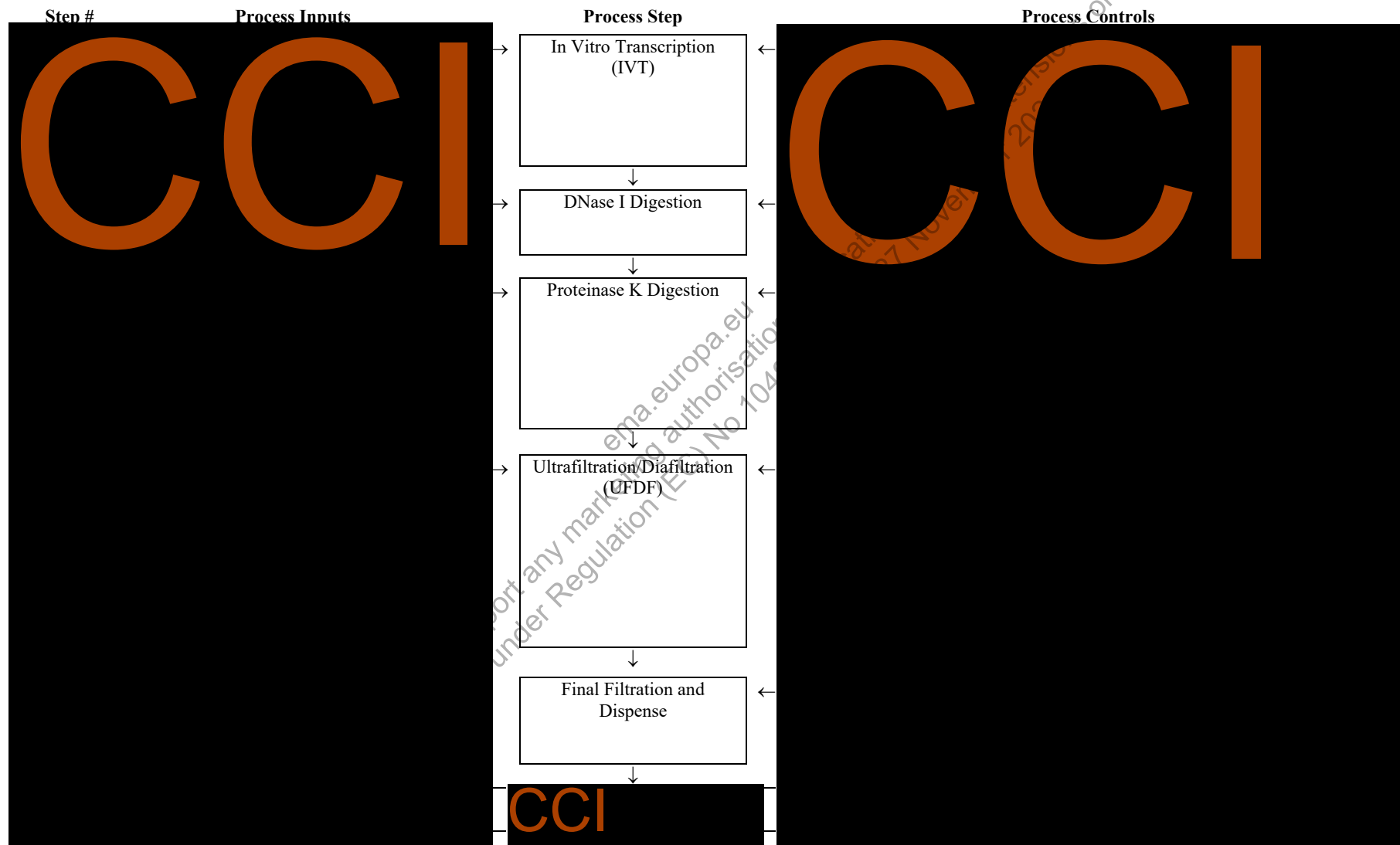
2.3.S.2.2.1. Overview of Manufacturing Process [Andover]

This section includes the description of the manufacturing process for BNT162b2 RNA drug substance. The RNA is first synthesized via an in vitro transcription (IVT) followed by DNase I and proteinase K digestion steps, which aid in purification. The crude RNA is then purified through a 2-stage ultrafiltration/diafiltration (UFDF). Lastly, the RNA undergoes a final filtration before being dispensed and stored frozen.

A flow diagram for the drug substance process is shown in [Figure 2.3.S.2-1](#). Refer to [Section 3.2.S.2.2 Description of Manufacturing Process and Process Controls \[Andover\]](#) for more detailed descriptions for each process step.

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Figure 2.3.S.2-1. RNA Manufacturing Process



2.3.S.2.2.1.1. In Vitro Transcription (IVT)

The primary objective of the IVT step is to synthesize RNA for drug substance production.

2.3.S.2.2.1.2. DNase I Digestion

The primary objective of the DNase I digestion step is to reduce the size of linear DNA template and enable subsequent removal across the ultrafiltration/diafiltration step.

2.3.S.2.2.1.3. Proteinase K Digestion

The primary objective of the proteinase K digestion step is to reduce the size of proteins in the reaction mixture for subsequent removal across the ultrafiltration/diafiltration step.

2.3.S.2.2.1.4. Ultrafiltration/Diafiltration (UFDF)

The UFDF step reduces small process-related impurities and concentrates and buffer exchanges the RNA into the final DS formulation.

2.3.S.2.2.1.5. UFDF Membrane Life Validation

The UFDF membrane lifetime will be established through at-scale concurrent validation studies that are currently ongoing.

2.3.S.2.2.1.6. Final Filtration and Dispense

The UFDF pool undergoes a bulk final 0.45/0.2 µm filtration into a flexible container. Final drug substance release testing is performed at this stage. The drug substance (DS) is then dispensed into CCI [REDACTED]

2.3.S.2.2.1.7. Drug Substance Storage and Transportation

The DS FCs are frozen and stored between -15 °C and -25 °C. DS FCs shipments using an insulated shipper are qualified for a shipping time of up to 106 hours at temperatures ≤ -15 °C.

2.3.S.2.2.1.8. Bulk Final Refiltration Procedure

In the event that the post-use integrity test on the final CCI [REDACTED] filter fails, the bulk DS may be refiltered through an unused CCI [REDACTED] filter. The Bulk Filtration and Dispense Refiltration step is performed in the same manner as the initial Final Filtration and Dispense step.

2.3.S.2.2.2. Batch Scale and Definition [Andover]

Definition of a Production Batch – BNT162b2 Drug Substance

Commercial scale drug substance batches are executed CCI [REDACTED] starting volume for in vitro transcription (IVT). All material produced is purified by a single two-stage ultrafiltration/diafiltration (UFDF) to produce drug substance.

Batch Number System

The batch number system for drug substance consists of a material designation code followed by a unique number assigned by the material inventory system.

Each drug substance batch is assigned one batch number for the entire IVT-UFDF process. Batches are released under their drug substance batch number.

2.3.S.2.2.3. Overview of Manufacturing Process [BNT &Rentschler]

Section not provided.

2.3.S.2.2.3.1. Batch Scale and Definition [BNT &Rentschler]

Section not provided.

2.3.S.2.3. Control of Materials

Data for this section is pending and will be updated once the data has been generated, analyzed, and verified.

2.3.S.2.3.1. Materials Used in Manufacture [Andover]

A list of the materials used in the manufacture of BNT162b2 drug substance is given in section 3.2.S.2.3 Control of Materials – Materials Used in Manufacture [Andover]. The site of manufacture has a vendor management program including appropriate quality systems, to ensure control of raw materials used for GMP manufacturing. As a result, these materials are purchased from approved suppliers. The raw materials used in the drug substance manufacturing process are tested and released upon receipt in accordance with internal raw material specifications. Specifications for all non-compendial grade raw materials are described in Section 3.2.S.2.3.2.

Purified water or water for injection (WFI) manufactured at the facility is used throughout the drug substance process and meets USP/Ph. Eur. requirements.

2.3.S.2.3.2. Materials Used in Manufacture [BNT &Rentschler]

Section not provided.

2.3.S.2.3.3. Control of Materials - Source, History and Generation of Plasmids

2.3.S.2.3.3.1. Plasmid Used for Production of the Linear DNA Template

Manufacture of the BNT162b2 drug substance is achieved using in vitro transcription that includes a linear DNA template as a starting material. The linear DNA template is produced via plasmid DNA from transformed *Escherichia coli* cells. CCI

2.3.S.2.3.3.1.1. Plasmid Cell Bank and Linear DNA Template Manufacturer(s)

The cell bank manufacture and storage, starting material (linear DNA template) manufacture and associated testing are performed in the Pfizer facility at 875 Chesterfield Parkway West, Chesterfield MO 63017. The cell bank testing was performed at Charles River laboratory, Inc, 358 Technology Dr, Malvern, PA 19355. The cell bank sequence testing was performed at Genewiz, 115 Corporate Boulevard, South Plainfield, NJ 07080.

2.3.S.2.3.3.1.2. Plasmid Cell Banking System, Characterization and Testing

Plasmid cell banks have been prepared in accordance with ICH guideline: *ICH Q5D Derivation and characterization of cell substrates used for production of biotechnological/biological products*. Cell banking operations were performed in a controlled manufacturing area with appropriate precautions against adventitious contamination and cross-contamination from other cell lines.

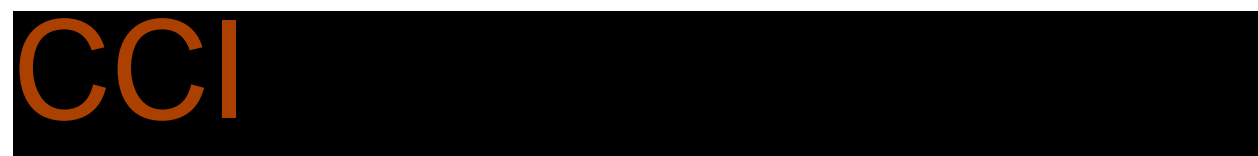
Preparation of pST4-1525 Master Cell Bank



Release testing of the Plasmid Master Cell Bank

Culture purity and identity testing performed on the plasmid MCB DW8968 provide confirmation that the cell bank is free from microbial and bacteriophage contamination and is of an E. coli lineage. The studies were designed in accordance with ICH Q5D guidelines.

Preparation of pST4-1525 Working Cell Bank





Release testing of the Plasmid Working Cell Bank

Culture purity and identity testing performed on the plasmid WCB DW8970 provide confirmation that the cell bank is free from microbial and bacteriophage contamination and is of an *E. coli* lineage. The studies were designed in accordance with ICH Q5D guidelines.

2.3.S.2.3.3.1.3. Preparation, Qualification and Storage of Renewal Plasmid Working Cell Banks (WCBs)

Renewal plasmid working cell banks (WCBs) will be prepared by expanding cells thawed from the MCB DW8968. WCB manufacturing operations will be performed in a controlled manufacturing area with appropriate precautions against adventitious contamination and cross-contamination from other cell lines.

The process steps for WCB preparation are described in [3.2.S.2.3 Control of Materials-Materials Used in Manufacture \[Andover\]](#). All steps of the WCB preparation process are documented in a manufacturing batch record.

Qualification of Renewal Working Cell Banks

Tests for identity, purity, and bacteriophage contamination will be performed to confirm the acceptability of renewal WCBs. In addition, future WCBs will be analyzed to demonstrate genotypic and plasmid integrity consistent with the MCB. The acceptance criteria for WCB qualification are summarized in 3.2.S.2.3 Control of Materials – Materials Used in Manufacture [Andover].

Plasmid Cell Bank Stability Testing

The plasmid MCBs and WCBs are enrolled in a cell bank stability program consisting of viability and plasmid retention assays conducted at all stability time points. The stability testing timepoints occur at defined intervals beginning from the cell bank release date (time zero) and subsequently at 24 months, 48 months, 72 months, and then every five years until the cell bank is depleted or no longer used for manufacturing.

2.3.S.2.3.3.2. Linear DNA Template Manufacturing

Cells from the WCB are thawed and the culture is expanded in shake flasks, which are then used to inoculate the fermenter. The culture medium used for expansion and fermentation

CCI is free of animal-derived components. Following fermentation, the cells are harvested and chemically lysed to recover the plasmid DNA. After this lysis step, the circular plasmid DNA is purified by ultrafiltration/diafiltration and chromatography.

Following purification, the circular plasmid DNA is incubated with a restriction enzyme, CCI, in order to linearize the plasmid followed by ultrafiltration/diafiltration.

The linear DNA template is filtered and dispensed in a qualified biosafety cabinet in a qualified cleanroom in order to ensure the final linear DNA template is free of any adventitious agent. The linear DNA template manufacturing process contains no components of animal origin.

The circular plasmid DNA and linear DNA template are tested to the specifications outlined in 3.2.S.2.3 Control of Materials – Materials Used in Manufacture [Andover] and the linear DNA template is used as a starting material in the drug substance manufacturing.

2.3.S.2.3.3.3. Linear DNA Template Specifications

The release specifications for the linear DNA template are given in 3.2.S.2.3. Control of Materials - Source, History and Generation of Plasmids, Table 3.2.S.2.3-10. The analytical control strategy includes sampling and testing of a selected number of attributes prior to linearization and the remainder of the attributes on the final linear DNA template.

2.3.S.2.3.3.4. Linear DNA Template Batch Analysis

The batch analysis data for representative lots of the linear DNA template are given in 3.2.S.2.3. Control of Materials - Source, History and Generation of Plasmids. At the time of manufacture of these batches, the commercial specifications had not yet been determined.

2.3.S.2.4. Control of Critical Steps and Intermediates

2.3.S.2.4.1. Control of Critical Steps and Intermediates – Manufacturing Process

Data for this section is pending and will be updated once the data has been generated, analyzed, and verified.

Process parameters discussed in this section include all critical process parameters (CPP). Critical process parameters (CPPs) were conservatively defined after the Cause and Effect (C&E) risk assessment by evaluating the parameters which had a strong functional relationship to a quality attribute (high C&E scores) as supported by data available to date from the process characterization studies, established scientific rationale or platform knowledge.

Assessment of the methodology for the determination of critical process parameters is provided in Section 3.2.S.2.6 Process Risk Assessment Strategy.

For assessment of the RNA drug substance process, the assignment of parameter criticality was expanded for all applicable quality attributes.

In-process test for control (IPT-C) and in-process test for monitoring (IPT-M) are used throughout the process to ensure consistent manufacturing. IPT-Cs are in-process tests used to control a quality attribute within a specified range so that it meets the desired DS/DP quality.

IPT-Ms are in-process tests used to monitor a quality attribute to either ensure that it is consistent with respect to previous process history or for forward processing. The monitoring tests may have action limits. These IPT-Cs and IPT-Ms are described in [Section 3.2.S.2.4 In-Process Test Methods \[Andover\]](#).

These in-process controls (process parameters and in-process tests) are used to ensure control of the individual process steps, process consistency and product quality. If the results of these controls are outside of the acceptable ranges/acceptance criteria, an evaluation of the deviation is performed and the material could subsequently be dispositioned for further manufacture, based on the investigation conclusion.

Section 3.2.S.2.4 In-Process Test Methods [Andover] provides a list of in-process controls (critical process parameters and IPT-Cs) with their acceptable ranges/acceptance criteria for the drug substance manufacturing process.

2.3.S.2.4.2. Control of Critical Steps and Intermediates - In-Process Test Methods [Andover]

2.3.S.2.4.2.1. In-Process Testing for Control (IPT-C)

2.3.S.2.4.2.1.1. Determination of RNA Concentration by Ultraviolet (UV) Spectroscopy

The UV spectroscopy method is used to quantitate the RNA concentration in the ultrafiltration/diafiltration (UFDF) pool (pre- or post-dilution) and is used to determine UFDF step yield. Details of the analytical procedure are described in [Section 3.2.S.4.2. UV Spectroscopy](#). A summary of the method validation is detailed in [Section 3.2.S.4.3 UV Spectroscopy](#).

2.3.S.2.4.2.2. In-Process Testing for Monitoring (IPT-M)

Descriptions of the in-process test methods for monitoring are provided below. These IPT-Ms with established action limits are used to routinely monitor the manufacturing process and ensure that the process remains in a state of control.

2.3.S.2.4.2.2.1. Determination of RNA Concentration by Ultraviolet (UV) Spectroscopy

The UV spectroscopy method is used to quantitate the RNA concentration in the proteinase K pool sample to determine IVT yield and at various steps during the UFDF recovery operation. Details of the analytical procedure and the corresponding method validation are provided in Section 3.2.S.4.2 UV Spectroscopy and Section 3.2.S.4.3 UV Spectroscopy, respectively.

2.3.S.2.4.2.2.2. Bioburden

The bioburden procedure is performed to determine the microbial load of viable microorganisms in the **CC**

CCI The analytical procedure is performed following the principles described in the local compendia, USP <61>, Ph. Eur. 2.6.12, and JP 4.05 using membrane filtration methodology.

2.3.S.2.4.2.2.3. Endotoxin

The purpose of bacterial endotoxin testing is to measure the level of bacterial endotoxins in the **CCI**

CCI. The analytical kinetic turbidimetric limulus amoebocyte lysate (LAL) procedure is performed following the principles described in the local compendia, USP <85>, Ph. Eur. 2.6.14, and JP 4.01.

2.3.S.2.4.3. Control of Critical Steps and Intermediates - In-Process Test Methods [BNT Mainz & Rentschler]

Section not provided.

2.3.S.2.4.4. Control of Critical Steps and Intermediates – Hold Times [Andover]

The validated hold times for manufacturing are described in Table 2.3.S.2-2. Pool hold times are not required for routine processing, but strategic holds in the process (≥ 24 h) to aid in manufacturing scheduling were validated as described in [Section 3.2.S.2.5 Hold Times \[Andover\]](#).

Table 2.3.S.2-2. Hold Times for mRNA Drug Substance (Routine Manufacturing)

CCI

2.3.S.2.4.5. Control of Critical Steps and Intermediates – Hold Times [BNT Mainz & Rentschler]

Section not provided.

2.3.S.2.5. Process Validation/Evaluation

2.3.S.2.5.1. Process Validation/Evaluation- Overview [Andover]

The validation of the drug substance manufacturing was conducted for independent, consecutive batches. The validation of the drug substance production process was designed to provide documented evidence that the manufacturing process, when operating within defined process controls, would consistently produce drug substance meeting pre determined acceptance criteria and demonstrate expected, reproducible, and consistent process performance.

The control of the process was demonstrated by maintaining process parameters within the defined limits specified in the manufacturing batch records and process validation (PV) protocols.

The process performance assessments included as part of the validation of the drug substance production process are listed below.

2.3.S.2.5.1.1. Process Validation and or Evaluation - Validation of Removal of Impurities [Andover]

During process development and manufacture of the BNT162b drug substance, the manufacturing process has successfully been shown to effectively and consistently deliver drug substance with acceptable levels of process and product related impurities and potential contaminants.

The panel of process and product related impurities and potential contaminants listed in [3.2.S.2.5 Process Validation and/or Evaluation - Removal of Impurities \[Andover\]](#) will be further evaluated during process validation and was selected to demonstrate that the manufacturing process can consistently deliver drug substance with an acceptable level of impurities. These impurities will be controlled through drug substance release specifications and are discussed in detail in [Section 3.2.S.3.2 Impurities](#).

2.3.S.2.5.1.2. Process Validation and or Evaluation - Manufacturing Process [Andover]

Data for this section is pending and will be updated once the data has been generated, analyzed, and verified.

The purpose of this study was to demonstrate consistency of the in vitro transcription (IVT), DNase I digestion, proteinase K digestion, ultrafiltration diafiltration, final filtration and dispense unit operations for the drug substance manufacturing process for the first three process performance qualification (PPQ)/ process validation (PV) batches against the PPQ acceptance criteria.

The effective and consistent removal of process-derived impurities was demonstrated for the BNT162b2 drug substance manufacturing process during process validation. These data are reported and discussed in [Section 3.2.S.3.2 Impurities](#).

The drug substance validation batch release testing results are provided in [Section 3.2.S.4.4 Batch Analysis](#). All results met the pre-determined acceptance criteria.

2.3.S.2.5.1.3. Process Validation and or Evaluation - Validation of In-Process Test Methods [Andover]

Data for this section is pending and will be updated once the data has been generated, analyzed, and verified.

2.3.S.2.5.1.3.1. Endotoxin

The Limulus Amebocyte Lysate (LAL) bacterial endotoxin analytical procedure, using the kinetic turbidimetric LAL method, was validated for the quantitative determination of the

level of bacterial endotoxins in BNT162b2 in-process samples. The bacterial endotoxins analytical procedure was performed in alignment with the current United States Pharmacopeia (USP) Chapter <85>, Ph. Eur. 2.6.14, and JP 4.01.

2.3.S.2.5.1.3.2. Bioburden

The validation of the bioburden analytical procedure (performed following the principles described in USP <61>, Ph. Eur. 2.6.12, and JP 4.05) for BNT162b2 drug substance (DS) was performed based on guidance from USP <1227>. The method validation (challenge recovery test) challenges the test method to ensure that the test articles are non-inhibitory to the recovery of inoculated microorganisms.

2.3.S.2.5.1.4. Process Validation and/or Evaluation - Hold Times [Andover]

Data for this section is pending and will be updated once the data has been generated, analyzed, and verified.

Multiple hold time studies have been performed for the drug substance process to establish the maximum duration of holds for in-process pools. These studies were designed to demonstrate effective microbiological control of bioburden and endotoxin and product quality at both normal routine processing as well as at maximum allowable batch record limits.

In-process pool hold times are not required for routine processing, but strategic holds in the process ≥ 24 hours to aid in manufacturing scheduling were validated. Periods of less than 24 hours are considered active processing times and are not required to be evaluated unless product is shown to be unstable.

Data presented in this section will demonstrate microbial hold data from media simulation studies using the containers at commercial scale and biochemical stability for in-process pools at small scale to support the validation of in-process holds. Biochemical stability for durations <24 hours during routine processing were also evaluated as part of the study and the conditions are summarized in Table 2.3.S.2-3.

Table 2.3.S.2-3. Validated Maximum In-Process Hold Times



2.3.S.2.5.1.5. Process Validation and/or Evaluation - Filter Qualification and Validation [Andover]

Data for this section is pending and will be updated once the data has been generated, analyzed, and verified.

Filter qualifications were executed to demonstrate that the indirect and direct process intermediate contact filters used in the BNT162b2 drug substance manufacturing process met the requirements set forth in the qualification protocol.

The requirements evaluated during filter qualification include operational performance requirements as well as verification of vendor data.

The filter types and classifications for production of drug substance are listed in [3.2.S.2.5 Process Validation and/or Evaluation - Filter Qualification and Validation \[Andover\]](#) and summarize the points of use across all manufacturing steps in the process.

2.3.S.2.5.1.6. Process Validation and/or Evaluation - Shipping Performance Qualification [Andover]

[Section 3.2.S.2.5 Process Validation and/or Evaluation - Shipping Performance Qualification \[Andover\]](#) summarizes the data generated to support the successful qualification of the shipping process for transport of frozen BNT162b2 drug substance CCI at a temperature of $\leq 15^{\circ}\text{C}$ during the air and ground shipments from the drug substance manufacturing site Pfizer, Andover, MA USA to the drug product manufacturing sites up to 106 hours. This leaflet covers multiple routes for the global supply chain.

The leaflet also covers the shipping qualification for the CCI that may be used in the future after appropriate qualifications during at-scale batch operations.

2.3.S.2.5.1.7. Process Validation and/or Evaluation - Additional Process Evaluation [Andover]

[Section 3.2.S.2.5 Additional Process Evaluation \[Andover\]](#) summarizes additional process evaluation studies such as ongoing concurrent membrane life validation at manufacturing scale and continued process verification.

2.3.S.2.5.2. Process Validation/Evaluation- Overview [BNT Mainz & Rentschler]

Section not provided

2.3.S.2.5.2.1. Process Validation and/or Evaluation - Validation of Removal of Impurities [BNT Mainz & Rentschler]

Section not provided

2.3.S.2.5.2.2. Process Validation and/or Evaluation - Manufacturing Process [BNT Mainz & Rentschler]

Section not provided

2.3.S.2.5.2.3. Process Validation and/or Evaluation - Validation of In-Process Test Methods [BNT Mainz & Rentschler]

Section not provided

2.3.S.2.5.2.4. Process Validation and/or Evaluation - Hold Times [BNT Mainz & Rentschler]

Section not provided

2.3.S.2.5.2.5. Process Validation and/or Evaluation - Filter Qualification and Validation [BNT Mainz & Rentschler]

Section not provided

2.3.S.2.5.2.6. Process Validation and/or Evaluation - Shipping Performance Qualification [BNT Mainz & Rentschler]

Section not provided

2.3.S.2.5.2.7. Process Validation and/or Evaluation - Additional Process Evaluation [BNT Mainz & Rentschler]

Sections not provided

2.3.S.2.6. Manufacturing Process Development

A scientific and risk based approach has been used to identify the vaccine critical quality attributes (CQAs), and develop drug substance and drug product manufacturing processes that consistently deliver the desired quality.

This manufacturing process development section for drug substance is organized as follows:

1. Quality Attributes
2. Process Risk Assessment Strategy
3. Process Development and Characterization
4. Risk Assessment of Process Related Impurities
5. Development History and Comparability
6. Control Strategy
7. Analytical Method Evolution

2.3.S.2.6.1. Quality Attributes

Quality attributes (QAs) of the BNT162b2 drug substance (DS) were identified and assessed for criticality. DS attributes were initially identified based on their potential relationship to product quality. The process initially considered the quality target product profile (QTPP) of

the BNT162b2 vaccine drug product and the potential impact of the attributes to safety and/or efficacy of the drug product. Compendial and regulatory expectations were also taken into consideration.

A summary of the quality attributes with the rationale for the criticality assignment is provided in [Section 3.2.S.2.6 Manufacturing Process Development – Quality Attributes](#).

2.3.S.2.6.2. Process Risk Assessment Strategy

A structured quality risk management program is utilized for all new products, which includes Cause and Effect Matrices (C&E) and Failure Modes and Effects Analysis (FMEA).

A C&E assessment is initially performed in order to prioritize higher risk process parameters for process characterization studies and other critical control strategies. The scoring for the initial C&E tables was performed based upon prior knowledge including process and platform understanding, manufacturing experience, and relevant public domain information.

Following an update to the C&E scores, FMEA tables are generated. The FMEA exercise ensures adequate attention is given to higher risk process parameters with respect to process control. The FMEA is used to evaluate independent process parameters for each step with respect to their level of control (occurrence and detection) and their potential to impact drug substance quality and yield (severity). Potential risk is quantitated via scoring for severity, occurrence and detection to give an overall risk priority number (RPN) as the output of the FMEA assessment. As part of the FMEA assessment, the higher risk process parameters are ranked in relation to RPN scoring and risk mitigation identified.

Please refer to [3.2.S.2.6 Manufacturing Process Development - Process Risk Assessment Strategy](#) for further details.

2.3.S.2.6.3. Process Development and Characterization

Data for this section is pending and will be updated once the data has been generated, analyzed, and verified.

This includes a discussion of the scale-down models as well as the approach, results and conclusions from the process characterization studies completed. This section also includes description of approaches taken to identify critical process parameters, as well as how the operating ranges have been defined in order to assure product quality (Section 3.2.S.2.6 Process Development and Characterization).

2.3.S.2.6.4. Risk Assessment of Process Related Impurities

Data for this section is pending and will be updated once the data has been generated, analyzed, and verified.

This includes a safety risk assessment for all the potential process related impurities included in the drug substance process relative to patient safety. These potential impurities include **CCI** (Section 3.2.S.2.6 Risk Assessment of Potential Process Related Impurities).

2.3.S.2.6.5. Development History and Comparability

Data for this section is pending and will be updated once the data has been generated, analyzed, and verified.

This also includes the chronological history of the batches and major changes during the production campaigns. The batches are compared in the comparability assessment with focus on critical quality attributes as well as heightened product characterization. The results indicate that the changes resulted in comparable or improved product quality (Section 3.2.S.2.6 Development History and Comparability Assessment).

2.3.S.2.6.6. Control Strategy

Data for this section is pending and will be updated once the data has been generated, analyzed, and verified.

This includes the approach to developing the control strategy as well as the summary for the control strategy for all quality attributes controls implemented and section references to where the controls are described in greater detail (Section 3.2.S.2.6 Control Strategy).

2.3.S.2.6.7. Analytical Method Evolution

The analytical testing strategy applied to BNT162b2 drug substance has evolved throughout the development history. These changes to the analytical testing strategy are summarized in [Table 2.3.S.2-4](#)

Table 2.3.S.2-4. Evolution of BNT162b2 Drug Substance Methods

Process		Clinical (Process 1)	Emergency Supply ^a (Process 2)	Process Performance Qualification, Commercial Supply (Process 2)
Quality Attribute	Analytical Procedure			
Clarity	Appearance	R	R	R, S
Coloration	Appearance	R	R	R, S
pH	Potentiometry	R	R	R, S
Osmolality	Osmometry	R	Not required	Not required
RNA sequence	Sequencing of DNA starting material	R	Not required	Not required
Content (RNA concentration)	UV Spectroscopy	R, S	R, S	R, S
Identity: RNA length	Agarose gel electrophoresis	R	Not required	Not required
Identity: as RNA		R	Not required	Not required
Identity of encoded RNA sequence	RT-PCR	Not required	R	R
RNA integrity	Capillary gel electrophoresis	R, S	R, S	R, S
5'-Cap	HPLC-UV	Not required	Not required	R, S
Poly(A) tail	ddPCR	Not required	Not required	R, S
Residual DNA template	qPCR	R	R	R
Residual double stranded RNA (dsRNA)	Immunoblot	R	R	R
Bacterial endotoxins	Endotoxin (LAL)	R	R	R, S
Bioburden	Bioburden	R	R	R, S

a. Emergency supply designation applies to U.S. market.

Abbreviations: R = Release; S = Stability; RT-PCR = reverse transcription polymerase chain reaction; ddPCR = droplet digital PCR; qPCR = quantitative PCR; dsRNA = double stranded RNA; LAL = Limulus amoebocyte lysate

2.3.S.3. CHARACTERISATION

2.3.S.3.1. Elucidation of Structure and Other Characteristics

[Section 3.2.S.3.1 Elucidation of Structure and Other Characteristics](#) describes the structure and characteristics of BNT162b2 drug substance (DS) which have been assessed using the analytical approaches outlined in Table 2.3.S.3-1. The analytical methodologies employed for BNT162b2 RNA drug substance characterization are capable of evaluating primary structure, including 5'-capping and 3'-poly(A) tail, and higher order structure. The results demonstrate that BNT162b2 RNA drug substance has the expected structure.

Analytical characterization was performed with BNT162b2 drug substance batch (20Y513C101).

Table 2.3.S.3-1. Characterization Strategy for BNT162B2 Drug Substance

Characteristic	Analytical Approach	Methodology	Section References
Primary structure	Confirm expected RNA sequence at the oligonucleotide level	Reversed phase HPLC-UV and tandem mass spectrometry (LC/MS/MS) of oligonucleotide fragments generated by RNase T1 digestion	Section 2.3.S.3.1.1.1
	Confirm expected RNA sequence at the oligonucleotide level	Illumina MiSeq Next Generation Sequencing Technology	Section 2.3.S.3.1.1.2
5'-Cap structure	Confirm the 5' capping structure and 5'-end profile	Reversed phase HPLC-UV and mass spectrometry (LC-UV/MS) analysis of purified 5' terminal after RNaseH digestion	Section 2.3.S.3.1.1.3
Poly(A)-tail	Confirm the presence and determine the length of poly(A)-tail	Reversed phase HPLC-UV and mass spectrometry (LC-UV/MS) analysis of purified poly(A)-tail after Ribonuclease T1 digestion	Section 2.3.S.3.1.1.4
Higher order structure (HOS)	Spectroscopic analysis to confirm the presence and fingerprint of HOS	Circular dichroism (CD) spectroscopy	Section 2.3.S.3.1.1.5

2.3.S.3.1.1. Primary Structure

2.3.S.3.1.1.1. LC/MS/MS - Oligonucleotide Mapping

The primary sequence of BNT162b2 DS was analyzed by LC/MS/MS - oligonucleotide mapping. BNT162b2 DS was digested with RNase T1, and the resulting enzymatic fragments were separated by ion-paired reversed-phase high performance liquid chromatography (IP-RP-HPLC) with UV detection at CCI [REDACTED]

[REDACTED]

[REDACTED]

The LC/MS/MS – oligonucleotide mapping results are summarized in Table 3.2.S.3.1-3 and demonstrate that BNT162b2 DS contains the correct sequence as predicted from the linear DNA template (Section 3.2.S.2.3 Control of Materials – Source, History and Generation of Plasmids).

Further details are provided in [Section 3.2.S.3.1 Elucidation of Structure and Other Characteristics](#).

2.3.S.3.1.1.2. Sequencing of RNA

In order to further confirm sequence identity, RNA sequencing for BNT162b2 DS was performed using the CCI [REDACTED]

[REDACTED] and the sequence identity was confirmed.

Taken together, the RNA sequencing results further demonstrate that the BNT162b2 transcript generated during the *in vitro* transcription (IVT) process bears the correct RNA sequence as predicted from the linear DNA template.

2.3.S.3.1.1.3. 5'-Cap Characterization by LC-UV/MS

The characterization of the 5' end capped (5'-Cap) and un-capped species of BNT162b2 DS was accomplished by ion-pair reversed-phase high performance liquid chromatography-ultraviolet light detection at CCI and online electrospray ionization mass spectrometry (RP-HPLC/UV-ESI MS) or LC-UV/MS. Sample handling and chromatography follow the method described in [Section 3.2.S.4.2 Reversed Phase – High Performance Liquid Chromatography \(RP-HPLC\)](#).

CCI

Further details are provided in [Section 3.2.S.3.1 Elucidation of Structure and Other Characteristics](#).

CCI

2.3.S.3.1.1.4. 3' Poly(A)-tail Characterization by LC-UV/MS

Analysis of the 3' polyadenosine tail (poly(A)-tail) of BNT162b2 DS was accomplished by ion-pair reversed-phase high performance liquid chromatography with UV detection at CCI and on-line electrospray ionization mass spectrometry (RP-HPLC-UV/ESI MS or LC-UV/MS). The poly(A)-tail of BNT162b2 DS was cleaved off by ribonuclease T1 (RNase T1) followed by isolation via CCI affinity purification.

Further details are provided in [Section 3.2.S.3.1 Elucidation of Structure and Other Characteristics](#).

The LC-UV/MS results demonstrate that BNT162b2 DS contains the expected poly(A)-tail

CCI

2.3.S.3.1.1.5. Higher Order Structure

The higher order structure of BNT162b2 mRNA DS was characterized in solution using

CCI

CCI

2.3.S.3.2. Impurities

Data for this section is pending and will be updated once the data has been generated, analyzed, and verified.

This section summarizes the impurities that are controlled and monitored during the BNT162b2 drug substance manufacturing process.

Commercial scale batch testing results demonstrate that the process is capable of effectively and consistently delivering drug substance with acceptable levels of process and product related impurities and potential contaminants listed are listed in this section. Process validation data collected to date is provided for each impurity and will continue to be updated as testing for additional process validation batches is complete.

2.3.S.3.2.1. Process-Related Impurities

Process-related impurities are defined as impurities that originate from the manufacturing process and may be derived from reagents used in the in-vitro transcription and purification processes.

The process related impurities discussed in this section include residual DNA template.

2.3.S.3.2.2. Residual DNA Template

Residual DNA template is a process-related impurity derived from the linearized DNA template added to the in-vitro transcription reaction. Residual DNA template is further controlled through routine testing using the analytical procedure described in [3.2.S.4.2 Quantitative Polymerase Chain Reaction \(qPCR\)](#) and the BNT162b2 drug substance specification as described in [3.2.S.4.1 Specification](#).

2.3.S.3.2.3. Additional Process Related Impurities

The additional process related impurities that were evaluated include CCI. For further information about the evaluation of potential process-related impurities and result refer to [Section 3.2.S.2.6 Risk Assessment of Potential Process Related Impurities](#).

2.3.S.3.2.2. Product-Related Impurities

The product related impurities discussed in this section include double stranded RNA. Safety assessment of double stranded RNA was performed as part of specification setting. Refer to [3.2.S.4.5 Justification of Specifications](#) for information pertaining to levels of these impurities relative to patient safety.

Double Stranded RNA

Double stranded RNA is a product-related impurity derived from the in-vitro transcription reaction. Double stranded RNA is further controlled through routine testing using the analytical procedure described in [3.2.S.4.2 Immunoblot](#) and the BNT162b2 drug substance specification as described in [3.2.S.4.1 Specification](#).

Potential Contaminants

Potential contaminants are defined as any adventitiously introduced materials (e.g., chemical, biochemical, or microbial species) not intended to be part of the manufacturing process of the drug substance or drug product (ICH Q6B).

The potential contaminants that may be present in BNT162b2 drug substance are endotoxin and bioburden.

During manufacture of the BNT162b2 drug substance, the manufacturing process has successfully been shown to effectively and consistently deliver drug substance with acceptable levels of the process and product related impurities and potential contaminants.

2.3.S.4. CONTROL OF DRUG SUBSTANCE

2.3.S.4.1. Specification

The specification for BNT162b2 drug substance at release and during stability studies is provided in Table 2.3.S.4-1. The acceptance criteria are applicable from batch release to end of shelf-life. The acceptance criteria provided are based on the available data. These criteria will be reassessed and amended as appropriate when more data become available.

Table 2.3.S.4-1. BNT162b2 Drug Substance Specification

Quality Attribute	Analytical Procedure	Acceptance Criteria
Composition and Strength		
Clarity	Appearance (Clarity) ^a	CCI
Coloration	Appearance (Coloration) ^a	Not more intensely colored than level of the brown (B) color standard
pH	Potentiometry ^a	CCI
Content (RNA Concentration)	UV Spectroscopy	CCI
Identity		
Identity of Encoded RNA Sequence	RT-PCR ^b	Identity confirmed
Purity		
RNA Integrity	Capillary Gel Electrophoresis	CCI intact RNA
5'- Cap	RP-HPLC	CCI
Poly(A) Tail	ddPCR	CCI
Process Related Impurities		
Residual DNA Template	qPCR ^b	CCI
Product Related Impurities		
dsRNA	Immunoblot ^b	CCI
Safety		
Bacterial Endotoxin	Endotoxin (LAL) ^a	CCI
Bioburden	Bioburden ^a	CCI

a. Compendial

b. Assay not performed on stability.

Abbreviations: NTU = Nephelometric Turbidity Units; B = brown; RT-PCR = reverse transcription polymerase chain reaction; ddPCR = droplet digital PCR; qPCR = quantitative PCR; dsRNA = double stranded RNA; LAL = Limulus amoebocyte lysate; EU = endotoxin unit; CFU = colony forming unit

2.3.S.4.2. Analytical Procedures

Analytical procedures for the control of BNT162b2 drug substance, including those common to BNT162b2 drug substance and BNT162b2 drug product, are listed in [Table 2.3.S.4-2](#) and [Table 2.3.S.4-3](#).

Table 2.3.S.4-2. Analytical Procedures Common to BNT162b2 Drug Substance and BNT162b2 Drug Product

Analytical Procedure	Quality Attribute
Potentiometry	pH
RT-PCR	Identity of Encoded RNA Sequence
Capillary Gel Electrophoresis	RNA Integrity
Endotoxin	Bacterial Endotoxin

Abbreviations: RT-PCR = reverse transcription polymerase chain reaction

Table 2.3.S.4-3. Analytical Procedures for BNT162b2 Drug Substance Only

Analytical Procedure	Quality Attribute
Appearance	Clarity and Coloration
UV Spectroscopy	Content (RNA Concentration)
RP-HPLC	5'-Cap
ddPCR	Poly (A) Tail
qPCR	Residual DNA Template
Immunoblot	Residual dsRNA
Bioburden	Bioburden

Abbreviations: ddPCR = digital droplet polymerase chain reaction, dsRNA = double stranded RNA, qPCR = quantitative polymerase chain reaction, RP-HPLC = reversed phase-high performance liquid chromatography

Detailed information regarding analytical procedures is provided in [Section 3.2.S.4.2, Analytical Procedures - Overview](#).

2.3.S.4.3. Validation of Analytical Procedures

Validation of analytical procedures was performed to ensure the composition, strength, identity, purity, and safety of BNT162b2 drug substance. All non-compendial and compendial analytical procedures were confirmed suitable for their intended use.

Analytical procedures were validated against the parameters presented in ICH Q2(R1), Validation of Analytical Procedures: Text and Methodology, for their respective methodology categories. Quantitative analytical procedures were validated for precision, accuracy, specificity, linearity, range, and robustness. Quantitative procedures used to determine the content of minor constituents were further validated for quantitation limit (QL) and/or detection limit (DL). The identity analytical procedures were evaluated for specificity and robustness. Compendial procedures were verified for use in accordance with the applicable pharmacopeias.

Summaries of the non-compendial validations performed for BNT162b2 drug substance release and stability analytical procedures are provided in this section.

2.3.S.4.4. Batch Analyses

BNT162b2 drug substance batches used for nonclinical toxicology, clinical trials, process performance qualification (PPQ), emergency supply, and stability are summarized in [Table 2.3.S.4.4](#).

A full drug substance genealogy can be found in [Section 3.2.S.2.6 Developmental History and Comparability](#). The analytical testing strategy applied to BNT162b2 drug substance has evolved throughout the development history. Information on the drug substance method evolution/testing strategy is provided in [Section 3.2.S.2.6 Analytical Method Evolution](#).

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Table 2.3.S.4-4. Summary of COVID-19 Vaccine BNT162b2 Drug Substance Batches

Batch	Date of Manufacture	Production Site and Process ^a	Scale (L)	Purpose of Material	Data Table Location
RNA-RF200321-06	19-MAR-2020	BioNTech RNA Pharmaceuticals GmbH; Process 1	CC	Nonclinical toxicology, Stability	Table 3.2.S.4.4-2
R427-P020.2-DS	17-APR-2020	BioNTech Innovative Manufacturing Services GmbH (IMFS); Process 1		Clinical, Stability	Table 3.2.S.4.4-3
R438-P020.2-DS	29-MAY-2020	BioNTech Innovative Manufacturing Services GmbH (IMFS); Process 1		Clinical, Stability	Table 3.2.S.4.4-3
R443-P020.2-DS	30-JUN-2020	BioNTech Innovative Manufacturing Services GmbH (IMFS); Process 1		Clinical, Stability	Table 3.2.S.4.4-3
R445-P020.2-DS	24-JUL-2020	BioNTech Innovative Manufacturing Services GmbH (IMFS); Process 1		Clinical inventory, Stability	Table 3.2.S.4.4-3
20Y513C101	29-JUL-2020	Pfizer, Andover, MA, US; Process 2		Emergency supply ^b , Clinical inventory, Stability	Table 3.2.S.4.4-4
20Y513C201	13-AUG-2020	Pfizer, Andover, MA, US; Process 2		Emergency supply ^b , Stability	Table 3.2.S.4.4-4
20Y513C301	20-AUG-2020	Pfizer, Andover, MA, US; Process 2		Emergency supply ^b , Process performance qualification, Stability	Table 3.2.S.4.4-4
20Y513C401	27-AUG-2020	Pfizer, Andover, MA, US; Process 2		Process performance qualification, Stability	Table 3.2.S.4.4-4
20Y513C501	10-SEP-2020	Pfizer, Andover, MA, US; Process 2		Process performance qualification, Stability	Table 3.2.S.4.4-4

- a. See Section 3.2.S.2.6 Development History and Comparability Assessment for process description.
b. Emergency supply designation applies to U.S. market.

2.3.S.4.5. Justification of Specification

The specification for BNT162b2 drug substance is based on an understanding of the control strategy and CQAs for the drug substance. The attributes tested and associated acceptance criteria ensure the consistency of drug substance and linkage to clinical experience. This preliminary specification was established to ensure the quality, purity, potency/biological activity and safety of the commercial drug substance at release and during storage. The specification was informed by:

- Development experience (manufacture and analytical) with BNT162b2 drug substance;
- Total BNT162b2 manufacturing experience, including drug substance batches used to manufacture drug product lots used in nonclinical and clinical studies;
- The ongoing release and stability data for drug substance.

Currently, process performance qualification and associated characterization are ongoing and based on their outcome, the specification will be reassessed.

2.3.S.4.5.1. Specification-Setting Strategy

A comprehensive panel of analytical procedures has been implemented along with corresponding acceptance criteria to monitor and control BNT162b2 drug substance quality at release and over shelf life.

Appropriate analytical procedures were established to monitor and assess BNT162b2 drug substance as detailed in [Section 3.2.S.4.2 Analytical Procedures](#) and [Section 3.2.S.4.3 Validation of Analytical Procedures](#). With the exception of RT-PCR (identity), qPCR (residual DNA template) and Immunoblot (dsRNA) assays, which are conducted at drug substance release only, all other procedures are conducted at release and during stability studies for drug substance.

The approach to setting acceptance criteria for each quality attribute in the BNT162b2 drug substance specification included understanding gained from:

- Data obtained for drug substance batches used in the manufacture of nonclinical and clinical trial supplies.
- Experience with the analytical procedure and knowledge of the method capabilities. Release data for process 2 drug substance included in the evaluation and establishment of the commercial specification were generated with the validated analytical procedures outlined in [Section 3.2.S.4.2 Analytical Procedures](#).
- Comparability demonstrated across the development history. See [Section 3.2.S.2.6 Manufacturing Process Development-Development History and Comparability Assessment](#) for the comparability assessment of drug substance process 1 and process 2.

- The regulatory guidance for RNA-based products, where appropriate.
- Relevant BNT162b2 drug substance development data, including understanding of an impact to potency, safety and immunogenicity of the quality attribute evaluated, as well as the institutional experience with other mRNA products.

Because no appreciable stability data are yet available for representative BNT162b2 drug substance at the recommended storage condition of $-20 \pm 5^{\circ}\text{C}$, the acceptance criteria used for stability over shelf life will be the same as the acceptance criteria used for batch release.

Thus, the acceptance criteria in the drug substance specification reflect the current understanding of criticality of quality attributes, their impact on product performance, and the quality of the product used in clinical trials.

The analytical testing strategy applied to BNT162b2 has evolved during the development history for the molecule. Several drug substance analytical method changes have been made during product development, particularly in preparation for process 2 manufacture. 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 prior to process validation. In addition to the BNT162b2 product-specific methods, standard compendial test methods are performed in accordance with the current requirements.

Method evolution and changes, with bridging information as appropriate, are described in detail [Section 3.2.S.2.6 Analytical Method Evolution](#).

Please refer to [Section 3.2.S.4.5 Justification of Specification](#) for further information.

2.3.S.5. REFERENCE STANDARDS OR MATERIALS – PREPARATION AND CHARACTERIZATION OF REFERENCE MATERIAL

Drug substance reference material is being prepared for use as a reference material and/or assay control for the release and stability testing of drug substance and drug product clinical and process validation materials, as well as initial commercial supply.

A summary of the drug substance reference material is presented in Table 2.3.S.5-1.

A two-tiered system for in-house reference material will be implemented in the future to support the commercial product. After implementation, the primary reference material (PRM) will be intended to last throughout the commercial product lifetime and will be used for qualification of future working reference materials (WRM). PRM is not intended to be used in routine testing.

An initial WRM will also be made and implemented in the future to support routine testing of commercial drug substance and drug product until it is consumed and replaced.

Table 2.3.S.5-1 Summary of Reference Materials

Reference Material Designation	Parental Drug Substance Batch	Reference Material Establishment Date	Types of Material Released/Evaluated
Clinical Reference Material lot number 20Y513C201-RM	20Y513C201	September 2020	Clinical Supplies Process Validation Initial Commercial Supplies
Primary Reference Material	TBD	Planned for 2021	Working Reference Material
Working Reference Material	TBD	Planned for 2021	Commercial Supplies

NA = Not Applicable TBD = To be determined

2.3.S.6. CONTAINER CLOSURE SYSTEM

Data for this section is pending and will be updated once the data has been generated, analyzed, and verified.

2.3.S.6.1.1. CCI Containers

CCI



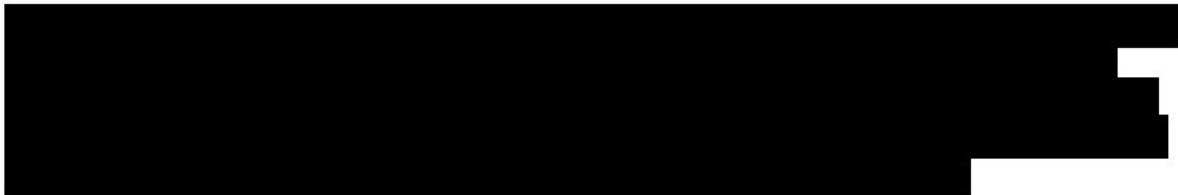
2.3.S.6.1.2. Extractables

CCI



2.3.S.6.1.3. Leachables

CCI



2.3.S.7. STABILITY

2.3.S.7.1. Stability Summary and Conclusions

The initial commercial shelf life of the BNT162b2 drug substance is 6 months when stored at the intended storage condition of $-20 \pm 5^{\circ}\text{C}$. The initial shelf life is based on the currently available data from stability studies utilizing material from three clinical drug substance batches manufactured using Process 1 and two clinical drug substance batches and three process validation batches manufactured using Process 2.

2.3.S.7.1.1. Stability Batches and Studies

The stability program is designed to follow ICH guidelines for stability of drug substance (ICH Guideline Q1A: Stability Testing of New Drug Substances and Products; ICH Guideline Q5C: Quality of Biotechnological Products, Stability Testing of Biotechnological/Biological Products). To date, three drug substance batches, 20Y513C301, 20Y513C401 and 20Y513C501, manufactured using the commercial process (Process 2) have been placed on stability and stored under long term, accelerated, and thermal stress conditions. These batches have been aliquoted into small volume containers, which are representative of the commercial CCI bags and stored under ICH conditions. The small volume containers represent a worse case with significantly higher surface area per unit solution volume compared to the large-scale commercial containers. Therefore, based on the use of the same product-contact materials of constructions CCI and a worse case dimensional ratio, the small volume containers are an appropriate scaled-down container closure system for use in the drug substance stability studies.

Two batches of clinical drug substance batches, 20Y513C101 and 20Y513C201, manufactured using the commercial process (Process 2) have been placed on stability and stored under long term, accelerated, and thermal stress conditions in small volume CCI bags. Stability data from these studies are considered to be predictive of the commercial drug substance.

Additionally, three clinical batches of drug substance have been manufactured using Process 1 and placed on stability and stored under long term, accelerated, and thermal stress conditions. These supportive stability batches of drug substance have been stored in polypropylene tubes which are commonly used for storing aliquots of aqueous solutions as they are inert plastics and were acceptable containers for early phase, supportive stability studies.

A summary of all drug substance batches on stability studies and current available stability data are shown in Table 2.3.S.7-1. These stability studies are currently on-going and data from these studies will be provided in the future and will be used to confirm the initial shelf life of the drug substance. Further information on confirmation and extension of the drug substance shelf life is discussed in Section 3.2.S.7.1. Stability Summary and Conclusions.

Table 2.3.S.7-1. Summary of On-going Stability Studies

Batch Number	Date of Manufacture	Batch Use	Study Type	Storage Condition	Available Data	Study Status
20Y513C501 (Pfizer, Andover)	September 2020	Process performance qualification, Stability	Long Term	-20 ± 5 °C	Release in progress	On-going
			Accelerated	5 ± 3 °C	Release in progress	On-going
			Thermal Stress	25 ± 2 °C/ 60 ± 5% RH	Release in progress	On-going
			Low Temperature	-90 to -60 °C	Release in progress	On-going
20Y513C401 (Pfizer, Andover)	August 2020	Process performance qualification, Stability	Long Term	-20 ± 5 °C	Release in progress	On-going
			Accelerated	5 ± 3 °C	Release in progress	On-going
20Y513C301 (Pfizer, Andover)	August 2020	Emergency supply ^a , Process performance qualification, Stability	Long Term	-20 ± 5 °C	T=0 (Release)	On-going
			Accelerated	5 ± 3 °C	T=0 (Release)	On-going
20Y513C201 (Pfizer, Andover)	August 2020	Emergency supply ^a , Stability	Long Term	-20 ± 5 °C	2 weeks	On-going
			Accelerated	5 ± 3 °C	2 weeks	On-going
			Thermal Stress	25 ± 2 °C/ 60 ± 5% RH	2 weeks	On-going
			Low Temperature	-90 to -60 °C	Release (T=0)	On-going
20Y513C101 (Pfizer, Andover)	July 2020	Emergency supply ^a , Clinical inventory, Stability	Long Term	-20 ± 5 °C	1 week	On-going
			Accelerated	5 ± 3 °C	1 month	On-going
			Thermal Stress	25 ± 2 °C/ 60 ± 5% RH	1 month	Complete
			Low Temperature	-90 to -60 °C	1 month	Complete
R443-P020.2-DS (BNT)	June 2020	Stability, Clinical	Long Term	-20 ± 5 °C	Release (T=0)	On-going
			Accelerated	5 ± 3 °C	Release (T=0)	On-going
			Thermal Stress	25 ± 2 °C	1 month	On-going
R438-P020.2-DS (BNT)	May 2020	Stability, Clinical	Long Term	-20 ± 5 °C	Release (T=0)	On-going
			Accelerated	5 ± 3 °C	Release (T=0)	On-going
			Thermal Stress	25 ± 2 °C	1 month	On-going
R427-P020.2-DS (BNT)	April 2020	Stability, Clinical	Long Term	-20 ± 5 °C	3 months	On-going
			Accelerated	5 ± 3 °C	3 months	On-going
			Thermal Stress	25 ± 2 °C	3 months	On-going

a. Emergency supply designation applies to U.S. market

2.3.S.7.1.2. Study Protocol for Drug Substance Batches at the Long Term Condition (-20 °C)

Aliquots of Pfizer manufactured drug substance batches have been stored at -20 ± 5 °C. Testing is being performed according to the protocol indicated in Table 2.3.S.7-2.

Table 2.3.S.7-2. Stability Protocol for Pfizer Drug Substance Primary Batches Stored at -20 ± 5 °C (Long Term Storage Condition)

Analytical Procedure	Test Intervals ^{a,c}
Appearance (Clarity)	0, 1W, 2W, 1M, 2M, 3M, 6M, 9M, 12M, 18M, 24M
Appearance (Coloration)	0, 1W, 2W, 1M, 2M, 3M, 6M, 9M, 12M, 18M, 24M
Potentiometry	0, 1W, 2W, 1M, 2M, 3M, 6M, 9M, 12M, 18M, 24M
Content (RNA Concentration) (UV Spectroscopy)	0, 1W, 2W, 1M, 2M, 3M, 6M, 9M, 12M, 18M, 24M
RNA Integrity (Capillary Gel Electrophoresis)	0, 1W, 2W, 1M, 2M, 3M, 6M, 9M, 12M, 18M, 24M
5'-CAP (RP-HPLC) ^b	0, 1W, 2W, 1M, 2M, 3M, 6M, 9M, 12M, 18M, 24M
Poly (A) Tail (ddPCR) ^b	0, 1W, 2W, 1M, 2M, 3M, 6M, 9M, 12M, 18M, 24M
Endotoxin (LAL)	0, 24M
Bioburden	0, 24M

- a. Initial data (t=0) are from release testing.
b. Testing performed only on primary batches
c. 1W, 2W and 2M testing not performed on 20Y513C301 and 20Y513C401
Abbreviations: W = Week, M = Month

Aliquots of BioNTech manufactured drug substance batches were stored at -20 ± 5 °C. Testing is being performed according to the protocol indicated in Table 2.3.S.7-3.

Table 2.3.S.7-3. Stability Protocol for BioNTech Drug Substance Batches Stored at -20 ± 5 °C

Analytical Procedure	Test Intervals (Months) ^a
RNA Content (UV Spectroscopy)	0, 3, 6, 9, 12, 18, 24
RNA Integrity (Capillary Gel Electrophoresis)	0, 3, 6, 9, 12, 18, 24

- a. Initial data (t0) are from release testing.

2.3.S.7.1.3. Study Protocol for Drug Substance Batches at the Accelerated Condition

To support manufacturing process, hold conditions and to study the effects of temporary excursions above the recommended storage conditions, drug substance aliquots have been stored at 5 ± 3 °C. Testing is being performed according to the protocol indicated in [Table 2.3.S.7-4](#) for the Pfizer manufactured drug substance batches and in [Table 2.3.S.7-5](#) for the drug substance batches manufactured by BioNTech.

Table 2.3.S.7-4. Stability Protocol for Pfizer Drug Substance Batches Stored at 5 ± 3 °C (Accelerated Condition)

Appearance (Clarity)	Test Intervals ^{ac}
Appearance (Coloration)	0, 1W, 2W, 1M, 3M, 6M
Potentiometry	0, 1W, 2W, 1M, 3M, 6M
Content (RNA Concentration) (UV Spectroscopy)	0, 1W, 2W, 1M, 3M, 6M
RNA Integrity (Capillary Gel Electrophoresis)	0, 1W, 2W, 1M, 3M, 6M
5'-CAP (RP-HPLC) ^b	0, 1W, 2W, 1M, 3M, 6M
Poly (A) Tail (ddPCR) ^b	0, 1W, 2W, 1M, 3M, 6M

- a. Initial data (t=0) are from release testing.
b. Testing performed only on primary batches
c. 1W and 2W not performed on 20Y513C301 and 20Y513C401
Abbreviations: W = Week, M = Month

Table 2.3.S.7-5. Stability Protocol for BioNTech Drug Substance Batches Stored at 5 ± 3 °C (Accelerated Condition)

Analytical Procedure	Test Intervals (Months) ^a
RNA Content (UV Spectroscopy)	0, 3, 6, 9, 12, 18, 24
RNA Integrity (Capillary Gel Electrophoresis)	0, 3, 6, 9, 12, 18, 24

- a. Initial data (t0) are from release testing.

2.3.S.7.1.4. Study Protocol for Drug Substance Batches at the Thermal Stress and Low Temperature Support Conditions

To support manufacturing process hold conditions and to study the effects of high temporary excursions from the recommended storage conditions, drug substance aliquots are being stored at 25 ± 2 °C and testing is being performed according to the protocol indicated in [Table 2.3.S.7-6](#) for drug substance batches manufactured by Pfizer and in [Table 2.3.S.7-7](#) for the drug substance batches manufactured by BioNTech.

Table 2.3.S.7-6. Stability Protocol for Pfizer Drug Substance Batches Stored at 25 ± 2 °C/ $60 \pm 5\%$ RH (Thermal Stress Condition)

Analytical Procedure	Test Intervals ^{ab}
Appearance (Clarity)	0, 1W, 2W, 1M
Appearance (Coloration)	0, 1W, 2W, 1M
pH	0, 1W, 2W, 1M
Content (RNA Concentration) (UV Spectroscopy)	0, 1W, 2W, 1M
RNA Integrity (Capillary Gel Electrophoresis)	0, 1W, 2W, 1M
5'-CAP (RP-HPLC)	0, 1W, 2W, 1M
Poly (A) Tail (ddPCR)	0, 1W, 2W, 1M

- a. Initial data (t0) are from release testing.
b. Testing not performed on 20Y513C301 and 20Y513C401
Abbreviations: W = Week, M = Month

Table 2.3.S.7-7. Stability Protocol for BioNTech Drug Substance Batches Stored at 25 ± 2 °C (Thermal Stress Condition)

Analytical Procedure	Test Intervals (Months) ^a
RNA Content (UV Spectroscopy)	0, 1, 3, 6
RNA Integrity (Capillary Gel Electrophoresis)	0, 1, 3, 6

- a. Initial data (t0) are from release testing.

To support manufacturing process hold conditions and to study the effects of low temporary excursions from the recommended storage conditions, drug substance aliquots of the batches manufactured by Pfizer are being stored at -90 to -60 °C. Testing is being performed according to the protocol indicated in Table 2.3.S.7-8.

Table 2.3.S.7-8. Stability Protocol for Pfizer Drug Substance Batches Stored at -90 to -60 °C (Low Temperature Support Condition)

Analytical Procedure	Test Intervals (months) ^{abc}
Appearance (Clarity)	0, 1M
Appearance (Coloration)	0, 1M
Potentiometry	0, 1M
Content (RNA Concentration) (UV Spectroscopy)	0, 1M
RNA Integrity (Capillary Gel Electrophoresis)	0, 1M
5'-CAP (RP-HPLC)	0, 1M
Poly (A) Tail (ddPCR)	0, 1M

- a. Initial data (t0) are from release testing.
b. One (1) week testing also performed on supportive batch 20Y513C101 for all tests but 5'-CAP and Poly(A) Tail.
c. Testing not performed on 20Y513C301 and 20Y513C401
Abbreviations: M=Month

2.3.S.7.1.5. Summary of Stability Data

2.3.S.7.1.5.1. Summary of Stability Data at the Long Term Storage Condition

Stability data from the batches stored at the long term condition of -20 ± 5 °C are presented in [Section 3.2.S.7.3 Stability Data - Long Term](#). Up to 3 months of data are currently available at this condition for batches manufactured by BioNTech and up to 1 months of data are currently available for batches manufactured by Pfizer. All data remained within the clinical acceptance criteria in place at the time of testing and the proposed commercial acceptance criteria, where applicable.

2.3.S.7.1.5.2. Summary of Stability Data at the Accelerated Condition

Stability data from the batches stored at the accelerated condition of 5 ± 3 °C are presented in [Section 3.2.S.7.3 Stability Data - Accelerated](#). Up to 3 months of data are currently available at this condition for batches manufactured by BioNTech and up to 1 months of data are currently available for batches manufactured by Pfizer. All data remained within the clinical acceptance criteria in place at the time of testing and the proposed commercial acceptance criteria, where applicable.

2.3.S.7.1.5.3. Summary of Stability Data at the Thermal Stress Condition

Stability data from the batches stored at the thermal stress condition of 25 ± 2 °C are presented in [Section 3.2.S.7.3 Stability Data - Thermal Stress](#). Up to 1 month of data are available for one batch manufactured by Pfizer (study has completed) and up to 2 weeks of data are currently available for a second batch manufactured by Pfizer. Release testing is on-going for additional batches manufactured by Pfizer. Additionally, there is up to 3 months of data available for the three batches manufactured by BioNTech. Out of specification RNA Integrity results were generated at the 1 month timepoint for Pfizer batch 20Y513C101 and the 3 month time point for BioNTech batch R427-P020.2-DS when stored at the thermal stress condition of 25 ± 2 °C. All other data generated to date remained within the clinical acceptance criteria and the proposed commercial acceptance criteria, where applicable. It is not unexpected to generate out of specification results for stressed stability conditions and this demonstrates the stability indicating properties of the analytical methods, therefore the out of specification results do not impact the overall stability study.

Stability data from the batches stored at the low temperature support condition of -90 to -60 °C are presented in [Section 3.2.S.7.3 Stability Data - Thermal Stress](#). Up to 1 month of data are available for one stability batch manufactured by Pfizer (study has completed) and release data are currently available for an additional two batches manufactured by Pfizer. All data remained within the clinical acceptance criteria in place at the time of testing.

A minimum of one process validation drug substance batch will be subjected to thermal cycling studies at a future date. These studies have not yet been initiated.

2.3.S.7.1.5.4. Summary of Stability Data at the Photostability Storage Condition

A minimum of one process validation drug substance batch will be subjected to the ICH photostability condition (option 2) at a future date. This study has not yet been initiated.

2.3.S.7.1.6. Conclusions for Shelf Life and Storage

The initial shelf life for the BNT162b2 drug substance is 6 months when stored at the recommended temperature of $-20 \pm 5^{\circ}\text{C}$ in CCI bags.

The initial shelf life is based on:

- Up to 3 months of current available stability data generated using drug substance manufactured using Process 1
- Up to 1 month of current available stability data generated using drug substance manufactured using Process 2
- Comparability demonstrated between Process 1 and Process 2 drug substance
- Understanding of the mRNA platform to support the initial shelf life

These stability studies are currently on-going and data from these studies will be provided in the future and will be used to confirm the initial shelf life of the drug substance, as well as extend the shelf life based on the acceptability of the data.

The shelf life will be extended beyond the 6 month initial shelf life using real time stability data on a minimum of 3 batches of commercially representative material. The sponsor will extend the assigned shelf life without notification providing the real time stability data at the intended storage condition is acceptable and within commercial specifications.

Additional drug substance batches representative of the commercial process may be placed on stability in the future. Protocols and data will be submitted in the future and used as additional support of the drug substance shelf life.

2.3.S.7.2. Post-approval Stability Protocol and Stability Commitment

Upon completion of the ICH stability protocols, post-approval, a minimum of one batch of BNT162b2 drug substance manufactured will be enrolled in the commercial stability program at the long term storage conditions of $-20 \pm 5^{\circ}\text{C}$ each year that drug substance is manufactured. The protocol for storage at $-20 \pm 5^{\circ}\text{C}$ in CCI bags is provided in

[Table 2.3.S.7-9](#).

Table 2.3.S.7-9. Post-Approval Commercial Stability Protocol for BNT162b2 Drug Substance Stored at -20 ± 5 °C in CCI Bags

Analytical Procedure	Test Intervals (Months) ^a
Appearance (Clarity)	0, 6, 12, 18, 24
Appearance (Coloration)	
pH	
UV Spectroscopy (Content (RNA Concentration))	
Capillary Gel Electrophoresis (RNA Integrity)	
5'-CAP (RP-HPLC)	
Poly (A) Tail (ddPCR)	0, End of shelf life
Bioburden	
Endotoxin	

a. Additional test intervals may be included for the purpose of extending expiry.

2.3.S.7.3. Stability Data

Stability data for long term conditions, accelerated condition, thermal stress and photostability studies are provided in Section 3.2.S.7.3.Stability Data.