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3.2.P.2.3. PROCESS DEVELOPMENT AND CHARACTERIZATION

3.2.P.2.3.1. Process Development and Characterization Studies for Unit Operations

This section describes the process development and characterization studies performed to understand the effect of process parameters on BNT162b2 product quality attributes. As both BNT162b2 and BNT162b1 were under clinical development at the same time, it was considered whether available data on BNT162b1 could provide additional support for manufacture of BNT162b2 drug product. Some process development and stability studies were performed using BNT162b1. Since the difference between the constructs is the length of the mRNA (BNT162b2 RNA: 4283 nucleotides; BNT162b1 RNA: 1262 nucleotides) it is reasonable that once encapsulated in the LNPs, freezing and temperature stress might be expected to impact LNP size, LNP polydispersity and % RNA encapsulation in a similar fashion for both constructs. As discussed in [Section 3.2.P.2.2 Drug Product](#), Section 3.2.P.2.2.1.3.2 BNT162b2 Drug Product Development Stability, BNT162b1 and BNT162b2 show similar physicochemical and colloidal stability under the same storage conditions. However, other attributes related to BNT162b1 RNA integrity or potency may not be representative of BNT162b2. Therefore, physicochemical and colloidal data as available for the BNT162b1 drug product are in some cases discussed here in lieu of or in addition to data for BNT162b2.

The initial process including LNP fabrication and drug product formulation, designated as the Research process, was developed at Acuitas Therapeutics, Vancouver, BC, Canada. A stage-appropriate scaled process was then developed in two phases at Acuitas Therapeutics, based on the same mixing apparatus configuration and using the same principles as the research scale process. During the first phase of the scale up process development (referred to as the Classical process), the changes involved introduction of pumps more suitable to higher flow rates and the implementation of conventional tangential flow filtration for the buffer exchange in order to manage the larger intermediate volumes at larger scales. The second phase of the scale up process (referred to as the Upscale process) focused on increasing the mass throughput of the RNA and lipids through the mixing device, in order to increase the batch size by six-fold in comparison to the Classical process. Suitability of the scaled manufacturing process was demonstrated with representative formulations in other programs. Transfer of the scaled manufacturing process for RNA/LNPs with the ALC-0315 formulation has been performed for manufacturing of animal and clinical trial material (using the Classical process) and emergency supply of the vaccine (using the Upscale process) at Polymun Scientific Immunbiologische Forschung GmbH. The commercial supply of the vaccine is achieved through a scale out approach in manufacturing of the LNPs. Scale out process is defined as the use of more than one T-mixer for LNP formation (i.e. 2-8 T-mixers) depending on the batch size, which was implemented at Polymun and at Pfizer Puurs and Kalamazoo.

The ongoing process development and characterization studies represent a combined experience derived both from laboratory scale studies using scale-down models and scaled-up manufacturing experience. A global approach to development is being undertaken across multiple manufacturing facilities in order to maximize vaccine production and availability with a highly aligned drug product manufacturing process across all sites and

manufacturing lines. The process as currently defined ([Section 3.2.P.3.3 Overview](#)) is supported by clinical manufacturing experience and manufacturing trials performed at scale for the lipid nanoparticle (LNP) manufacturing process as well as fill/finish of the final drug product. While not all manufacturing facilities are intended to be registered in all markets, the high degree of alignment in the manufacturing process design across all sites enables process understanding from development at one facility to be directly relevant to development for the process to be executed in other facilities. Process performance qualification (PPQ) manufacturing will confirm that the developed process is appropriate as defined for clinical and commercial manufacture.

The drug product commercial manufacturing process is outlined below and fully described in Section 3.2.P.3.3 LNP Fabrication and Bulk Drug Product Formulation and Section 3.2.P.3.3 Fill and Finish.

LNP Fabrication and Bulk Drug Product Formulation

- Drug substance thaw – Thawing of BNT162b2 drug substance
- Dilution of drug substance – Dilution with water and mixing of BNT162b2 thawed drug substance
- Preparation of organic phase – Dissolution of lipids in ethanol
- Lipid nanoparticle formation and stabilization – In-line dilution of water-diluted drug substance with citrate buffer to form aqueous phase; mixing of aqueous phase and organic phase in T mixer(s); dilution in-line of T-mixer output with citrate buffer
- Concentration, buffer exchange and filtration – Diafiltration, concentration, diafiltration, final concentration; 0.2 µm bioburden reduction filtration
- Concentration adjustment and addition of cryoprotectant – Concentration adjustment with PBS, addition of sucrose solution resulting in bulk drug product

Fill and Finish

- Sterile filtration - Bulk drug product 0.2 µm sterile filtration
- Aseptic filling - Aseptic filling into sterile glass vials, stoppering, capping
- Visual inspection
- Labeling and freezing
- Storage, packaging and shipment of BNT162b2 drug product

Characterization studies in support of various process steps took a holistic approach to evaluate physicochemical stability, considering the impact of various factors such as

formulation stage, contact materials, and temperature on quality attributes unrelated to microbial control. Microbial control is considered on a facility basis via media fill simulation.

Best practice guidance is that sampling and testing for bioburden and endotoxin should be performed prior to 0.2 µm filtration and at any step with hold time ≥24 hours. The process was reviewed and a risk-based approach was taken to address process steps that fall within this guidance (3.2.P.3.4 LNP Fabrication and Bulk Drug Product Formulation and 3.2.P.3.4 Fill and Finish).

Hold times for the commercial process will be confirmed based on PPQ. There are four steps in the drug product process (LNP Fabrication and Fill and Finish) that were tentatively assigned hold times ≥24 hours:

- **Drug Substance Thaw:** The hold time for this step is 4.2 1st ind [REDACTED]. This step is being further evaluated as there is no port on the EVA bags for sampling after thaw. The drug substance process includes bioburden and endotoxin testing at release which ensures that the drug substance is within specification for bioburden and endotoxin and thus is low risk for the drug product process, even with a hold time of 4.2 1st ind [REDACTED].
- **Concentration, Buffer Exchange and Filtration:** PBS buffer is held at 4.2 1st ind [REDACTED]. This buffer is sampled for bioburden and endotoxin prior to bioburden reduction filtration and use in this process step. In addition, the diafiltered and concentrated LNPs are passed through a bioburden reduction filter upon completion of this step.
- **Concentration Adjustment and Addition of Cryoprotectant:** As in the previous step, PBS buffer is held at 4.2 1st ind [REDACTED]. This buffer is sampled for bioburden and endotoxin prior to bioburden reduction filtration and use in this process step. The sucrose solution though held for 4.2 1st ind [REDACTED] is sampled for bioburden and endotoxin prior to use in this step.
- **Sterile Filtration:** Prior to redundant sterile filtration, the bulk drug product is sampled for bioburden and endotoxin. This step is included in the hold time for Drug Product (liquid) in vessels or glass vials at 4.2 1st ind [REDACTED] until the start of freezing.

Overall, the process is well controlled and appropriately tested for bioburden and endotoxin starting from the drug substance and ending with sterile filtration and fill/finish.

Process development to support operations related to drug substance is discussed in [Section 3.2.S.2.6 Process Development and Characterization](#).

Process development studies to support the drug product process include studies designed to understand the thawing of the drug substance, mixing of the diluted drug substance and the lipid components in the T-mixer for formation of the lipid nanoparticles (LNP), followed by

tangential flow filtration (TFF) for removal of process-related components and formulation of the LNP drug product, followed by sterile filtration, filling, capping and visual inspection. Freezing studies were also performed to support the freezing and storage operations of the final drug product which is stored at the recommended temperature of -90 °C to -60 °C. Studies are presented as follows:

- Hold times and compatibility of drug substance and drug product with process contact materials ([Section 3.2.P.2.3.2](#))
- Drug substance storage and thaw in ethylene vinyl acetate (EVA) bags ([Section 3.2.P.2.3.3](#))
- Dilution and mixing of drug substance ([Section 3.2.P.2.3.4](#))
- Preparation of the organic phase ([Section 3.2.P.2.3.5](#))
- Lipid nanoparticle formation and stabilization ([Section 3.2.P.2.3.6](#))
- Concentration, buffer exchange, and bioburden reduction filtration ([Section 3.2.P.2.3.7](#))
- Concentration adjustment and addition of cryoprotectant ([Section 3.2.P.2.3.8](#))
- Sterile filtration ([Section 3.2.P.2.3.10](#))
- Aseptic filling ([Section 3.2.P.2.3.11](#))
- Stoppering, sealing and capping ([Section 3.2.P.2.3.12](#))
- Visual inspection ([Section 3.2.P.2.3.13](#))
- Labeling and freezing of drug product ([Section 3.2.P.2.3.14](#))
- Drug product shipping ([Section 3.2.P.2.3.17](#))

A control strategy has been developed for the commercial process and is presented in [Section 3.2.P.2.3 Control Strategy](#).

As part of process development, the process was evaluated in a risk assessment. Cause and effect matrices were used to help identify process parameters that required further investigation. For each step in the manufacturing process, process parameters were identified. The potential impact of a deviation in each process parameter on each QA was assessed based on likelihood and severity criteria. The outcome of this assessment was a list of the most important process parameters throughout the entire manufacturing process. Based on the results of this risk assessment, studies were prioritized and performed in the development laboratory or at the commercial manufacturing sites. The parameters and quality attributes outlined in [Table 3.2.P.2.3-1](#) were evaluated during the respective process step by lab-scale studies or during engineering runs, process qualification and/or PPQ. Quality attributes were

chosen as those most relevant (most likely to be impacted) by the parameters being varied for evaluation in each step. Results from lab-scale studies and studies performed at the manufacturing sites as appropriate are presented here. Since the drug product has been manufactured by essentially the same process throughout clinical and into commercial manufacture, historical information, especially related to product quality, is available and was leveraged to develop the commercial process.

Table 3.2.P.2.3-1. Manufacturing Process Characterization Strategy

Process Step	Experimental Plan	Parameters Evaluated or To Be Evaluated	Quality Attributes Evaluated/Analytical Method
Drug Substance Thaw	4.2 1st ind		Appearance RNA Identity and Integrity/Agarose gel electrophoresis ^a RNA Integrity/CGE RNA Content/Fluorescence Bioburden pH/Potentiometry Osmolality Residual DNA template/qPCR dsRNA/Immunoblot Bacterial Endotoxins
Dilution of Drug Substance	4.2 1st ind		LNP Size and Polydispersity/DLS N/P ratio (calculated using amine groups in ALC-0315 (N) and phosphates (P) from backbone of RNA drug substance) ^a Potency/Mouse immunogenicity ^a RNA Integrity/CGE RNA Content and RNA Encapsulation/Fluorescence LNP Size and Polydispersity/DLS In Vitro Expression/Cell-based flow cytometry ^b
Preparation of Organic Phase			
Lipid Nanoparticle Formation and Stabilization			
Concentration, Buffer Exchange and Filtration			
Concentration Adjustment and Addition of Cryoprotectant			
Sterile Filtration	4.2 1st ind		RNA content and RNA Encapsulation/Fluorescence LNP Size and Polydispersity/DLS

Table 3.2.P.2.3-1. Manufacturing Process Characterization Strategy

Process Step	Experimental Plan	Parameters Evaluated or To Be Evaluated	Quality Attributes Evaluated/Analytical Method
Aseptic Filling	4.2 1st ind		N/A
Visual Inspection	4.2 1st ind	N/A	N/A
Labeling and Freezing	4.2 1st ind		LNP Size and Polydispersity/DLS RNA Encapsulation/Fluorescence In Vitro Expression/Cell-based flow cytometry ^b RNA integrity/CGE Poly(A) Tail/ddPCR ^b
Storage, Packaging and Shipment of BNT162b2 Drug Product	4.2 1st ind		pH/Potentiometry RNA Content and RNA Encapsulation/Fluorescence assay LNP Size and Polydispersity/DLS RNA Integrity/CGE In Vitro Expression/Cell-based flow cytometry ^b Potency/Mouse Immunogenicity ^a
All process steps from receipt of drug substance at the manufacturing site to the final freeze	4.2 1st ind		DS RNA Concentration/UV-vis pH/Potentiometry RNA Integrity/CGE LNP Size and Polydispersity/DLS Appearance/Visual RNA Content and RNA Encapsulation/Fluorescence In Vitro Expression/Cell-based flow cytometry ^b

a. Not a release and/or stability assay

b. Additional test for drug product

c. To date, Upscale process manufacturing has ranged from 4.2 1st ind drug substance for Emergency Supply and up to 4.2 1st ind for Engineering.

Abbreviations: CGE = capillary gel electrophoresis; DS = drug substance; LNP = lipid nanoparticles; TFF = tangential flow filtration; DLS = dynamic light scattering; N/P = nitrogen/phosphate ratio; N/A = not applicable; F/T = freeze/thaw

3.2.P.2.3.2. Hold Times

While it is recognized that the range for some hold times during commercial manufacture will be limited by available media fill simulated process validation (establishing control from a microbial perspective, see Section 3.2.P.3.5 Validation of Aseptic Process by Media Fills), a holistic approach was taken to establish ranges for hold times based on the physicochemical stability of BNT162b2 and important process materials, e.g. lipids during the LNP fabrication process.

This approach also considered factors such as temperature in evaluating the stability of BNT162b2 at various stages through the manufacturing process, including time out of freezer for product movement between freezers or during packaging. Data from laboratory-scale light exposure studies, formal stability studies and full-scale manufacturing experience during engineering runs contributed to determination of hold times for the different process steps from a product stability perspective. Hold times are confirmed during PPQ manufacturing.

3.2.P.2.3.2.1. Stability of Lipids Solubilized in Ethanol

A study was performed to evaluate the stability of lipids ALC-0135, ALC-0159, DSPC and cholesterol in absolute ethanol during solubilization at 4.2 1st ind and to support a process hold time at ambient temperature. Lipid mixtures were prepared in 4.2 1st ind

The study design is shown in Table 3.2.P.2.3-2. Lipid mixtures were incubated for 4.2 1st ind then filtered using a 0.2 µm filter into 4.2 1st ind. Samples were withdrawn after 4.2 1st ind and stored at 4.2 1st ind until analyzed.

Table 3.2.P.2.3-2. Study Design for Lipids Hold Time Support – Lipid Concentration

Lipids	Lot No.	Lipid Solutions			
		Exp. 1		Exp. 2	
		(mg)	4.2 1st ind	(mg)	4.2 1st ind
ALC-0315	WE:20-C0164	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind
ALC-0159	WE:20-C0048	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind
DSPC	WE:19-C0068	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind
Cholesterol	WE:19-C0188	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind

After the incubation and frozen storage, samples were analyzed by RP-HPLC-CAD for lipid integrity. Results as shown in Table 3.2.P.2.3-3 and Figure 3.2.P.2.3-1 for the 15 mg/mL solution and Table 3.2.P.2.3-4 and Figure 3.2.P.2.3-2 for the 30 mg/mL solution.

4.2 1st ind

Table 3.2.P.2.3-3. Lipid Integrity in Ethanol at 4.2 1st ind Mixture (µg/mL) (Experiment 1)

Lipids	T 0 h	T 3 h	T 6 h	T 24 h	T 48 h	T 72 h	T 96 h
ALC-0315	4.2 1st ind						
ALC-0159	4.2 1st ind						
DSPC	4.2 1st ind						
Cholesterol	4.2 1st ind						

Abbreviation: h = hour(s)

4.2 1st ind

4.2 1st ind

Table 3.2.P.2.3-4. Lipid Integrity in Ethanol at 4.2 1st ind Experiment 2)

Lipids	T 0 h	T 3 h	T 6 h	T 24 h	T 48 h	T 72 h	T 96 h
ALC-0315	4.2 1st ind						
DSPC	4.2 1st ind						
Chol	4.2 1st ind						
ALC-0159	4.2 1st ind						

Abbreviations: h = hour(s)

4.2 1st ind

4.2 1st ind

3.2.P.2.3.2.1.1. Conclusion

Based on the results of this study, the lipids are stable when dissolved in 100% ethanol and stored at 25 °C for up to 4 days.

3.2.P.2.3.2.2. Lipid Nanoparticle In-process Holds

During the LNP fabrication process the aqueous mRNA and organic lipids solutions are each pumped into a T-mixer at a prescribed flow rate, where the two streams meet and are mixed together. During this step the LNPs are formed. Once through the T-mixer the LNPs

and the LNPs are formulated with PBS and 300 mM sucrose at pH 7.4.

The drug product process developed by Polymun Scientific for manufacture of BNT162b2 for clinical supply is the basis of transfer to commercial production sites for commercial manufacture. At the commercial manufacturing sites, the length of time it takes to process the material may be longer than the smaller scale process performed at Polymun.

A development study was performed to determine appropriate LNP hold times at room temperature or 2-8 °C for the commercial manufacturing process. BNT162b2 drug substance (DS001426) (Table 3.2.P.2.3-5) was diluted with citric acid buffer and the 4 lipids were dissolved in ethanol.

Table 3.2.P.2.3-5. List of Materials for LNP Fabrication

Material	Lot number	Description
mRNA	20Y513C101	BNT162b2 Drug Substance Item no. DS001426
Cholesterol	700100-01-025	MFG: Avanti Polar Lipids Inc.
ALC-0315	850365-01-165	MFG: Avanti Polar Lipids Inc.
ALC-0159	970159-002	MFG: Avanti Polar Lipids Inc.
DSPC	850365-01-165	MFG: Avanti Polar Lipids Inc.

For this study, the LNP downstream process steps were interrupted by hold times as shown in Table 3.2.P.2.3-6.

Table 3.2.P.2.3-6. LNP Hold Time Study Experimental Design

Description	Buffer/Solvent Matrix	Length of Hold (hours)	Temperature (°C)
Hold quench overnight after making LNPs	4.2 1st ind	■	■
Time from beginning to completion of TFF ^a with additional hold to 12 hours	4.2 1st ind	■	■
Time from end of previous hold at RT with additional hold	4.2 1st ind	■	■
After bioburden reduction filtration at 2-8 °C batch held for additional total time at 2-8 °C	4.2 1st ind	■	■
LNPs diluted to target DP with addition of sucrose and additional hold	4.2 1st ind	■	■
Drug product filtered and stored at 2-8 °C until vialled and frozen	4.2 1st ind	■	■

a. TFF is performed at RT and typically takes 4.2 1st ind; for this experiment TFF time was 4.2 1st ind

b. The drug product was held at 4.2 1st ind until vialled.

Abbreviations: LNP = lipid nanoparticle; TFF = tangential flow filtration; PBS = phosphate-buffered saline; RT = room temperature; NA = not applicable

The control for this study was the same LNPs processed without the hold times. Samples from the control process and the final step from the process subjected to hold times were analyzed for size and polydispersity by dynamic light scattering, RNA content and encapsulation efficiency by fluorescence assay, RNA integrity by capillary gel electrophoresis (CGE) and in-vitro expression (IVE) by flow cytometry. Results of the analysis are shown in Table 3.2.P.2.3-7. IVE (%) for the two concentrations tested for the final hold sample were variable but this was attributed to assay variability. Results of all other LNP analysis suggests no difference between the control and final hold time study samples.

Table 3.2.P.2.3-7. Results of Analysis of Control and Final Sample from LNP Hold Time Study

Sample	RNA Integrity (%)	RNA Content (mg/mL)	Encapsulation Efficiency (%)	Size (nm)	Polydispersity	IVE Cells Positive (%)	
						4.2 1st ind	T
Control	4.2 1b	4.2 1b	4.2 1b	4.2 1b	4.2 1b	4.2 1b	4.2 1b
Final Hold Sample	4.2 1b	4.2 1b	4.2 1b	4.2 1b	4.2 1b	4.2 1b	4.2 1b

a. Sample size

Abbreviations: IVE = in vitro expression

3.2.P.2.3.2.2.1. Conclusion

Based on this hold time experiment, where lipid nanoparticles were held for different times and at different temperatures throughout completion of the manufacturing process, the data show that the particle attributes do not change over the course of the study. Acceptable hold times for LNPs during the manufacturing process are shown in Table 3.2.P.2.3-8.

Table 3.2.P.2.3-8. Acceptable Hold Times during LNP Manufacture

Description	Length of Hold (hours)	Temperature (°C)
Hold LNPs in quench matrix	4.2 1b	4.2 1b
Time from beginning to completion of TFF with additional hold	4.2 1b	4.2 1b
Time from end of previous hold at RT with additional hold	4.2 1b	4.2 1b
Batch hold after bioburden reduction filtration at 2-8°C	4.2 1b	4.2 1b
LNPs diluted to target DP with addition of sucrose	4.2 1b	4.2 1b

Abbreviations: LNP = lipid nanoparticle; TFF = tangential flow filtration; RT = room temperature

3.2.P.2.3.2.3. Laboratory Scale Product Contact Materials Compatibility and Light Exposure Studies

3.2.P.2.3.2.3.1. Product Contact Materials Compatibility

Product contact and light exposure studies provide important support for hold times. Product contact materials studies are not planned in the development laboratory as contact materials for commercial manufacture (filtration membranes/housings/tubing/tanks) are comparable to those used for clinical manufacture and have therefore been demonstrated to be appropriate through manufacturing experience. No effect on LNP colloidal stability or RNA content has been observed in release testing results for drug product manufactured for clinical supply, and testing results during preparation for commercial manufacturing have not shown any effects suggestive of adsorption or denaturation related to material compatibility.

3.2.P.2.3.2.3.2. Light Exposure Study

A light exposure study was performed in the development laboratory to monitor the stability of the drug substance and drug product using lighting conditions as measured at each of the Pfizer commercial sites at Kalamazoo, MI, USA and Puurs, Belgium. Although not all

facilities may be registered in all markets, information for both facilities is presented as relevant to overall process and product understanding. The study included process steps from receipt of drug substance through final freeze of the drug product vials at 4.2 1st ind For details on the commercial process steps, see Section 3.2.P.3.3 LNP Fabrication and Bulk Drug Product Formulation and Section 3.2.P.3.3 Fill and Finish.

Light intensities were measured for the planned manufacturing areas at the Kalamazoo and Puurs facilities. 4.2 1st ind

4.2 1st ind

(Table 3.2.P.2.3-9). Time while frozen and while in stainless steel tanks was considered non-exposure and was not included in the processing times. However, time that the drug product is packaged inside the translucent boxes before freezing was counted as fully exposed to ambient light.

Table 3.2.P.2.3-9 Kalamazoo and Puurs Facilities Cumulative Light Exposures for Development Study

Process Area	Visible Light Exposure				UV Light Exposure		
	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind
Drug Substance							
Thawing	4.2 1st ind						
Drug Product							
Formulation	4.2 1st ind						
Filling	4.2 1st ind						
Inspection	4.2 1st ind						
Labeling							
Packaging							
Freezing							

a. Used to determine the light exposure times for the study.

Abbreviations: mWh = milliWatt hours

BNT162b2 drug substance (DS) (Lot 20-DS-00037) was aliquoted 4.2 1st ind

4.2 1st ind

The test samples were stored frozen (-20 °C for DS and -70 °C for DP) until shipped on dry ice for analytical testing. Quality attributes and analytical tests performed are listed in Table 3.2.P.2.3-10. Results are compared to the protocol acceptance criteria. Encapsulation efficiency was also calculated for drug product.

Table 3.2.P.2.3-10. Analytical Testing for Light Exposure Study

Attribute	Test Material	Assay	Protocol Acceptance Criteria
DS RNA Concentration	DS	UV-Vis	4.2 1st ind
pH	DS	Potentiometry	7.0±0.5
	DP		7.4±0.5
RNA Integrity	DS	Capillary Gel Electrophoresis	4.2 1st ind
	DP		
LNP Size	DP	DLS	4.2 1st ind
LNP Polydispersity			4.2 1st ind
Subvisible Particles	DP	HIAC ultra-low volume	Meets compendial requirements
Appearance	DP	Appearance (Visual and Particles)	White to off-white suspension Essentially free from visible particulates
DP RNA Content	DP	Fluorescence assay	4.2 1st ind
In Vitro Expression	DP	Cell-based flow cytometry	Report results

Abbreviations: DS = drug substance; DP = drug product; LNP = lipid nanoparticle; DLS = dynamic light scattering

3.2.P.2.3.2.3.2.1. Sample Exposure Times and Light Intensity

The actual exposure times, and the measured and cumulative visible and UV light intensities for samples submitted for analytical testing are shown in Table 3.2.P.2.3-11 for drug substance and Table 3.2.P.2.3-12 for drug product. 4.2 1st ind

Table 3.2.P.2.3-11. Drug Substance Sample Exposure Times and Intensity Measurements

Timepoint (Hours)		Visible		UV	
		Measured	Cumulative	Measured	Cumulative
Planned	Actual	Lux	Lux-hours	mW/cm ²	mWh/cm ²
4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind
4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind
4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind
4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind
4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind
4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind
4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind

Table 3.2.P.2.3-12. Drug Product Sample Exposure Times and Intensity Measurements

Timepoint (Hours)		Visible		UV	
Planned	Actual	Measured Lux	Cumulative Lux-hours	Measured mW/cm ²	Cumulative mWh/cm ²

3.2.P.2.3.2.3.2. Drug Substance and Drug Product Light Exposure Testing Results

The actual exposure times and results of analytical testing are shown in Table 3.2.P.2.3-13 for drug substance and Table 3.2.P.2.3-14 for drug product.

Results of pH testing of all drug substance samples, including the control (T0) and the covered controls, were between 6.3 and 6.6. The T0 sample was at the lower end of the acceptance criteria for pH and some test samples were below the acceptance limit, but there was no trend. This suggests these results were not due to light exposure. RNA integrity (%) and concentration (mg/mL) results were all within the acceptance criteria for test samples and covered controls. There were no trends suggestive of any light exposure effects up to 4.2 1st ind for drug substance.

Table 3.2.P.2.3-13. Analytical Test Results for Drug Substance Exposed to Visible and UV Light

Sample	Actual Time (Hours)	pH	RNA Integrity (%)	RNA Concentration, (mg/mL)

Testing of the drug product exposed to visible and UV light at stated intensities for up to 4.2 1st ind showed essentially no difference from the T0 control or covered controls for pH, LNP size (nm) and polydispersity, RNA concentration (mg/mL) and encapsulation efficiency (%).

Integrity of the messenger RNA (mRNA) in the drug product samples was within the range 4.2 1st ind with essentially no difference between the light-exposed samples and covered controls. After 4.2 1st ind of light exposure both the test sample and the covered control were below the lower acceptance limit of 4.2 1st ind for RNA integrity. However, the T0 sample was at the low end of the acceptable range with 4.2 1st ind RNA integrity. The small difference between

4.2 1st inc

4.2 1st ind

This pattern was attributed to variability of this cell-based changes were not attributed to light exposure.

UV Light

Response	Percentage
Government should do more to protect the environment	4.2%

Abbreviations: EFVP = essentially free of visible particles; PDI = polydispersity index; EE = encapsulation efficiency; IVE = in vitro expression

4.2 1st ind

Table 3.2.P.2.3-15. Results of Drug Product Subvisible Particle Testing

Sample	4.2 1st ind	
Specification	4.2 1st ind	
Test-1: Samples Diluted with Formulation Buffer		
4.2 1st ind		
Test-2: Sample Diluted with Formulation Buffer		
4.2 1st ind		

Abbreviations: NMT = Not more than

3.2.P.2.3.2.3.2.3. Conclusions

The results of this study demonstrate that the maximum manufacturing times in ambient light determined for the Kalamazoo and Puurs commercial sites are acceptable with respect to UV and visible light exposure as there was no effect on the drug substance and the drug product quality attributes attributable to light exposure under the conditions tested.

- Cumulative visible light exposures were 4.2 1st ind for drug substance and 4.2 1st ind for drug product. The cumulative UV exposure for both drug substance and drug product was 4.2 1st ind.
- There was no effect on BNT162b2 drug substance or drug product attributable to light exposure under the conditions of this study.
- Based on results of this study, the suggested maximum exposure time of drug substance to ambient light conditions at Kalamazoo is 4.2 1st ind and at Puurs is 4.2 1st ind.
- Using weighted allotment for the formulation, filling and ILPF process steps, the maximum supported time for ambient light exposure is 4.2 1st ind at Puurs based on both visible and UV light conditions.
- The maximum supported time for ambient light exposure is 4.2 1st ind at Kalamazoo based on visible light exposure as Kalamazoo reported 4.2 1st ind UV exposure.
- Suggested process times based on this study are shown in Table 3.2.P.2.3-16.

Table 3.2.P.2.3-16. Suggested Process Times Based on Light Exposure Study

Process	Hold Time (Hours)		Visible						UV					
	Total	Allotted (%)	Tested Exposure (Lux-hrs)	KZO		Puurs		Tested Exposure (mWh/cm ²)	KZO		Puurs		Intensity (mW/cm ²)	Hrs
				Intensity (Lux)	Hours	Intensity (Lux)	Hours		Intensity (mW/cm ²)	Hrs	Intensity (mW/cm ²)	Hrs		
DS	4.2 1st ind													
DP			4.2 1st ind					4.2 1st ind						
Formulation	4.2 1st ind													
Fill	4.2 1st ind													
ILPF	4.2 1st ind													

Abbreviations: KZO = Kalamazoo; ILPF = inspection/labeling/packaging/freezing

3.2.P.2.3.2.4. Drug Product Hold Times for Packing, Shipping and Point of Use

The purpose of this hold time study was to evaluate the impact of cumulative temperature cycling on BNT162b2 drug product based on potential time out of condition (TOC) covering manufacturing, transportation and period of use (PoU) requirements. The desired conditions outside of long-term storage (-90 to -60 °C) without an impact on the critical drug product attributes are 14 days at 2-8 °C, 3 days at 25 °C, and >3 freeze/thaw (F/T) cycles.

A development lot of BNT162b2 LNPs formulated into bulk drug product (BDP) was used for the study. 4.2 1st ind [REDACTED] the BDP was filled into 2 mL vials at 0.45 mL/vial, stoppered and capped. The drug product at T0 was then subjected to the cumulative test conditions as shown in Table 3.2.P.2.3-17.

Table 3.2.P.2.3-17. Study Design for Packing, Shipping and Point of Use Hold Time Support

Material	Storage Temperature	Storage Time	Action	Sample	
				Name	Number
Formulated LNPs	NA	NA	NA	Bulk Drug Product	1
Bulk Drug Product	4.2 1st ind [REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	2
Drug Product	4.2 1st ind [REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	3
	4.2 1st ind [REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	4
	Freeze/Thaw Cycling – 4 Cycles				
	4.2 1st ind [REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	5
	[REDACTED]	[REDACTED]	[REDACTED]	4.2 1st ind [REDACTED]	6
	4.2 1st ind [REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	7
	[REDACTED]	[REDACTED]	[REDACTED]	4.2 1st ind [REDACTED]	8
	4.2 1st ind [REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	9

a. Included in calculation of 4.2 1st ind [REDACTED]
Abbreviations: LNP = lipid nanoparticle; F/T = freeze/thaw

Quality attributes were evaluated using the assays shown in Table 3.2.P.2.3-18. Particle size and size distribution were measured on the same day at the end of each step. The samples for the remaining assays were frozen at -80 °C until thawed for analysis. In addition, a sample from the last step of the study was tested for IgG response in a mouse model to evaluate the impact of the cumulative temperature cycling on the immunogenicity of the drug product.

Table 3.2.P.2.3-18. BNT162b2 Analytical Assays for Evaluation of Time Out of Condition Study Samples

Quality Attribute	Analytical Procedure	Acceptance Criteria
pH	Potentiometry	7.4±0.5
RNA Content	Fluorescence Assay	4.2 1st ind
Encapsulation Efficiency		4.2 1st ind
LNP Size	DLS	4.2 1st ind
Polydispersity		4.2 1st ind
RNA Integrity	CGE	4.2 1st ind
In Vitro Expression (IVE)	Cell-based Flow Cytometry	Report result

Abbreviations: LNP = lipid nanoparticle; DLS = dynamic light scattering; CGE = capillary gel electrophoresis

Analytical results for size and polydispersity by DLS were slightly increased from the T0 control but well within the acceptance criteria. RNA integrity by CGE, pH and RNA encapsulation efficiency by fluorescence assay were within the acceptance criteria and essentially unchanged from the T0 control at the last study timepoint. RNA content for the BDP (4.2 1st ind) by fluorescence assay was higher than the target 4.2 1st ind due to analytical measurement variation at the final LNP dilution step. The test samples ranged from 4.2 1st ind RNA but there was no trend indicative of any effect on RNA content by study conditions. The IVE assay results were variable but this variability was considered unrelated to the test conditions.

Table 3.2.P.2.3-19. Physicochemical Attributes and In Vitro Potency Testing Results for Time Out of Condition Study Samples

Sample		Size (nm)	PDI	pH	RNA Integrity (%)	RNA Content (mg/mL) ^a	RNA Encapsulation (%)	IVE (% Positive Cells)	
								150 ng	100 ng
1	4.2 1st ind								
2	4.2 1st ind								
3	4.2 1st ind								
4	4.2 1st ind								
5	4.2 1st ind								
6	4.2 1st ind								
7	4.2 1st ind								
8	4.2 1st ind								
9	4.2 1st ind								

Abbreviations: PDI = polydispersity index; IVE = in vitro expression; BDP = bulk drug product; DP = drug product; NT= not tested; F/T= freeze/thaw;

The results of the in vivo study indicated no significant difference in IgG response of the cumulative temperature cycled DP compared to clinical trial materials (CTMs) stored at 4.2 1st ind and observed at 4.2 1st ind post-vaccination in mice.

3.2.P.2.3.2.4.1. Conclusions

The results of this study indicated no change to the critical quality attributes of the drug product and therefore support these conditions outside of long-term storage (-90 to -60 °C)

- 14 days at 2-8 °C
- 3 days at 25 °C
- Up to 4 freeze/thaw cycles

3.2.P.2.3.2.5. Information from Formal Stability Studies

While development studies only consider physicochemical quality attributes, the formal stability studies are designed to follow ICH guidelines. BNT162b2 drug substance stored under long-term, accelerated, thermal stress, and temperature cycled conditions in EVA bags will be executed and summarized in [Section 3.2.S.7.1 Stability Summary and Conclusions](#).

Stability studies, designed to follow ICH guidelines, of BNT162b2 drug product stored under the recommended long-term, accelerated thermal cycling and photostability conditions will be executed and summarized in [Section 3.2.P.8.1 Stability Summary and Conclusion](#).

3.2.P.2.3.2.6. Full-Scale Manufacturing Experience

The stability of bulk drug product subjected to hold times in processing materials including stainless steel will be evaluated during PPQ and provided in Section 3.2.P.3.5 Hold Times.

Taken together, the information gathered from laboratory scale studies, formal stability studies, and full-scale manufacturing experience will confirm the proven acceptable range for hold times for commercial manufacturing.

3.2.P.2.3.3. Drug Substance Thaw

3.2.P.2.3.3.1. General Description

Drug substance (DS) is received from the drug substance manufacturing facility stored frozen in ethylvinylacetate (EVA) bags. Drug substance is stored in accordance with recommended storage conditions (Section 3.2.S.7.1 Stability Summary and Conclusions). Drug substance is then thawed in preparation for drug product manufacturing.

The frozen DS provided in EVA bags is thawed at 25 °C or controlled room temperature. The thaw time depends on heat transfer rates and fill volumes in the bags. At the end of the thaw process, the resulting solution is completely thawed (devoid of ice). Drug substance freezing and thawing may be conducted using either controlled freeze/thaw equipment consisting of an automated freeze/thaw unit and agitation platform capable of controlling the freezing and thawing rates (controlled rate freeze and controlled thaw) or utilizing a freezer (for freezing) or controlled temperature room (for thawing) (controlled room temperature thaw). Most manufacture of clinical supplies utilized a freezer for freezing and controlled room temperature for thawing of drug substance. 4.2 1st ind.

4.2 1st ind.

3.2.P.2.3.3.2. Characterization of Drug Substance Freezing and Thawing Operations

Operating conditions have been developed for thawing the drug substance in EVA bags, leveraging prior knowledge from Pfizer products similarly provided in EVA bags. Manufacturing scale data on BNT162b1 drug substance have been collected to demonstrate that the thawing process for the EVA bags results in a thawed drug substance solution, with no impact on product quality attributes. In addition to BNT162b2 clinical manufacturing experience, a freeze and thaw cycling study performed at scale using BNT162b2 directly supports the freezing and thawing of BNT162b2 drug substance and the resulting quality of the DP.

BNT162b1 drug substance (CZ07-P020.3-DS) was filled in EVA bags and subjected to freeze and thaw cycles. The filled EVA bags were frozen in a -20 ± 5 °C freezer and then thawed at room temperature, replicating worst case heat transfer rates. 4.2 1st ind.

. The product quality of the drug substance was analyzed and found to meet the acceptance criteria (Table 3.2.P.2.3-20). Although this study was performed with BNT162b1, the results are supportive of freeze-thaw cycles for BNT162b2 DS. This study indicated that the drug substance can be subjected to 4.2 1st ind. without impacting product quality. Product quality data for the BNT162b1 freeze-thaw study are shown in Table 3.2.P.2.3-20.

Table 3.2.P.2.3-20. Product Quality of BNT162b1 Drug Substance: Freeze and Thaw Study

Quality Attribute	Acceptable Range
Appearance (Clarity)	Clear 4.2 1st ind. colorless liquid
Integrity and identity (as RNA and RNA length)	(1) Single distinct band migrating at the expected location as compared to a length standard (2) No RNase-resistant band detectable by agarose gel electrophoresis
RNA Integrity	4.2 1st ind.
Content (RNA Concentration)	4.2 1st ind.
Bioburden	4.2 1st ind.
pH	4.2 1st ind.
Osmolality	4.2 1st ind.
Residual DNA template	4.2 1st ind.
Bacterial Endotoxin	4.2 1st ind.

Abbreviations: NTU = Nephelometry turbidity units; NT = Not tested; CFU = Colony-forming units

This study demonstrated that BNT162b1 drug substance is robust to a wide range of freezing and thawing process conditions. Based on this information, and the successful manufacture of BNT162b2 drug product for supply of clinical material, it was expected that the commercial process would be appropriate for BNT162b2 drug substance.

A study was then initiated with BNT162b2 drug substance using engineering batch CZ13-P020.2-DS. Flexible freeze/thaw (FFT) 4.2 1st ind. were filled with 4.2 1st ind. of drug substance and frozen at 4.2 1st ind. Bags were held for an additional 4.2 1st ind.

This study design was repeated for 4.2 1st ind. After 4.2 1st ind., samples were analyzed for appearance (Color and Clarity), pH, RNA concentration and integrity, Residual DNA template, double-stranded RNA (dsRNA), Bacterial endotoxins and Bioburden. Results are shown in Table 3.2.P.2.3-21. Results of the study demonstrated that BNT162b2 drug substance is essentially unchanged after 4.2 1st ind. of freeze and thaw which supports the parameters for drug substance thaw.

Table 3.2.P.2.3-21. Product Quality of BNT162b2 Drug Substance: Freeze and Thaw Cycling Study

Quality Attribute	Acceptance Criteria
Clarity (NTU)	4.2 1st ind.
Coloration	Not more intensely colored than level 1.2 of the B color standard
pH	4.2 1st ind.
Content (RNA concentration) (mg/mL)	4.2 1st ind.
RNA Integrity (%)	4.2 1st ind.
Residual DNA Template (ng DNA/mg RNA)	4.2 1st ind.
dsRNA (pg dsRNA/μg RNA)	4.2 1st ind.
Bacterial Endotoxins (EU/mL)	4.2 1st ind.
Bioburden (CFU/10 mL)	4.2 1st ind.

4.2 1st ind.

a. After cycle 4.2 1st ind.

b. RNA concentration below target is most likely a result of sampling heterogeneity as the result from 4.2 1st ind. at room temperature was performed before analysis.

Abbreviations: NTU = nephelometry turbidity units; b = Brown; N/T = not tested at that cycle; dsRNA = double stranded RNA; EU = endotoxin units; CFU = colony forming units

3.2.P.2.3.3.3. Conclusions

Manufacturing experience for clinical supply to date has included freezing of DS in a freezer set to maintain -20 ± 5 °C and thawing in a controlled temperature room (controlled room temperature thaw) in preparation for DP manufacture. For process efficiency, controlled freeze and thaw equipment is introduced for commercial manufacturing though thawing may be performed under controlled room temperature conditions. Multiple cycles of freeze and thaw are supported.

The thawing parameters for both controlled thaw and controlled room temperature thaw of drug substance are shown in Table 3.2.P.2.3-22 and Table 3.2.P.2.3-23. 4.2 1st ind.

Table 3.2.P.2.3-22. Process Parameters for Controlled Drug Substance Thaw

Process Parameter	Acceptable Range
4.2 1st ind.	
	4.2 1st ind.

a. Target set point

Abbreviation: HTF = heat transfer fluid

Table 3.2.P.2.3-23. Process Parameters for Controlled Room Temperature Drug Substance Thaw

Process Parameter	Acceptable Range
4.2 1st ind.	
	4.2 1st ind.

a. Thaw duration will be established based on the results of process qualification studies.

3.2.P.2.3.4. Dilution and Mixing of Drug Substance

3.2.P.2.3.4.1. General Description

Drug substance is transferred from one or more EVA bag(s) into the manufacturing tank. Water for injection is added to the tank to target a final RNA content 4.2 1st ind. and the solution is mixed.

3.2.P.2.3.4.2. Mixing for Dilution of Drug Substance

The parameters for mixing of the diluted drug substance are shown in Table 3.2.P.2.3-24. The setpoint range reflects Pfizer's extensive experience in drug product manufacture as well as the qualified range of the mixing vessels at the commercial manufacturing sites. 4.2 1st ind. during the process risk assessment and, during process validation manufacturing, 4.2 1st ind. will be evaluated from a homogeneity and product quality perspective.

Clinical manufacturing experience has also shown that in-process mixing (4.2 1st ind. [REDACTED]) and the final formulation (4.2 1st ind. [REDACTED]) were appropriate as drug product has been successfully manufactured for clinical trial material (Section 3.2.P.5.4 Batch Analyses).

3.2.P.2.3.4.3. Conclusions

The data obtained during process qualification and process performance qualification are expected to confirm that the mixing parameters used in the BNT162b2 drug product manufacturing process are appropriate to achieve homogeneity and that BNT162b2 is robust to the mixing shear stress experienced during the manufacturing process.

Table 3.2.P.2.3-24. Mixing Parameters for Dilution of Drug Substance

Process Parameter	Controlled Setpoint
4.2 1st ind. [REDACTED]	4.2 1st ind. [REDACTED]

a. To be confirmed during process validation based on equipment capability and fit to process.

3.2.P.2.3.5. Preparation of the Organic Phase

3.2.P.2.3.5.1. General Description

The organic phase is prepared by first thawing the lipids from -20 °C to 20±5 °C. Ethanol is added to the organic phase vessel and heated to improve dissolution of the lipids. The lipids are added to the organic phase vessel while mixing followed by a final q.s. with ethanol. The solution is mixed until lipids are fully dissolved at 4.2 1st ind. [REDACTED] for storage until use.

3.2.P.2.3.5.2. Development of the Organic Phase Preparation Process

The weights of the lipids and the ethanol for dissolution vary depending on batch size. 4.2 1st ind. [REDACTED] is important based on clinical manufacturing experience and will be monitored during process qualification and validation.

3.2.P.2.3.5.3. Conclusions

The process parameters for preparation of the organic phase are summarized in Table 3.2.P.2.3-25.

Table 3.2.P.2.3-25. Process Parameters for Preparation of Organic Phase

Process Parameter	Acceptable Range
4.2 1st ind.	Per unit formula ^a
	Per unit formula ^a
	Per unit formula ^a
	Per unit formula ^a
	Per unit formula ^a
	Per unit formula ^a
	4.2 1st ind.
	4.2 1st ind.

- a. Target set-point.
b. Mixing time will be established based on the results of process qualification studies.
c. Target range. Mixing temperature range to be confirmed during process qualification studies.

3.2.P.2.3.6. Lipid Nanoparticle (LNP) Formation and Stabilization

3.2.P.2.3.6.1. General Description

To form the LNPs, the citrate buffer is combined in-line with the diluted drug substance in a 4.2 1st ind. ratio to create the aqueous phase. The organic and aqueous phases are fed into a manifold of parallel T-mixer(s) to form the LNPs. 4.2 1st ind.

4.2 1st ind.

[REDACTED]

3.2.P.2.3.6.2. Development of the LNP Manufacturing Research Scale Process

4.2 1st ind.


[REDACTED]

[REDACTED] As BNT162b2 drug product is manufactured at multiple sites the storage temperature has evolved to incorporate qualified freezers in the range -90 °C to -60 °C which is the recommended storage temperature for commercial supply.

3.2.P.2.3.6.3. Development of LNP Manufacturing “Classical Process” and “Upscale Process”

A scaled-up process, termed “Classical Process” has been developed at Acuitas Therapeutics based on the same mixing apparatus configuration and using the same principles as the research scale process described above. Pumps more suitable to higher flow rates were introduced and conventional tangential flow filtration for the buffer exchange was implemented in order to manage the larger intermediate volumes at larger scales. Suitability of the scaled manufacturing process was demonstrated with representative formulations in other programs. The classical process, also referred to as “current” process, for manufacturing LNPs with ALC-0315 formulation was transferred to Polymun Scientific Immunobiologische Forschung GmbH, Austria for manufacturing of nonclinical animal trial material (ATM) and clinical trial material (CTM). Process performance has been verified by release data of ATM and CTM. Refer to [Section 3.2.P.5.4 Batch Analyses](#).

A further scaled LNP process, termed “Upscale Process,” was developed using BNT162b1 for manufacture of the LNPs to increase the mass throughput of drug substance 4.2 1st ind.



A comparison of the classical process and the upscale process is shown in Table 3.2.P.2.3-26. While the basic process has not changed for the LNP fabrication process, to enable larger scale manufacturing for emergency (applies to US market) and commercial supply, equipment and materials have been scaled up appropriately at the manufacturing sites to meet the projected batch sizes.

Table 3.2.P.2.3-26. Differences between Classical^a and Upscale Processes

4.2 1st ind.



a. Also referred to as “current” process
Abbreviations: DS = drug substance; LNP = lipid nanoparticle

The LNPs were evaluated by size, polydispersity, RNA encapsulation and the lipid to drug ratio (N/P). In addition, the topology of the LNPs was characterized by small angle X-ray scattering. The potency of LNPs from each process was determined as well by mouse immunogenicity studies.

As shown in Figure 3.2.P.2.3-3, [Figure 3.2.P.2.3-4](#), [Figure 3.2.P.2.3-5](#) and [Figure 3.2.P.2.3-6](#) the physicochemical properties and the N/P ratios of the LNPs produced in the upscale process (Upscale 1, Upscale 2) are comparable to those from the classical process as represented by DP lot BCV150620 and clinical trial materials (CTM). Solid and dashed lines indicate 3 and 1 standard deviations from the mean respectively.

Figure 3.2.P.2.3-3. Comparability of the Particle Size for LNPs Produced by the Classical Process and Upscale Process



Figure 3.2.P.2.3-4. Comparability of Polydispersity Index (PDI) for LNPs Produced by the Classical Process and Upscale Process



Figure 3.2.P.2.3-5. Comparability of Encapsulation Efficiency (%) for LNPs Produced by the Classical Process and Upscale Process



Figure 3.2.P.2.3-6. Comparability of the N/P Ratio for LNPs Produced by the Classical Process and Upscale Process



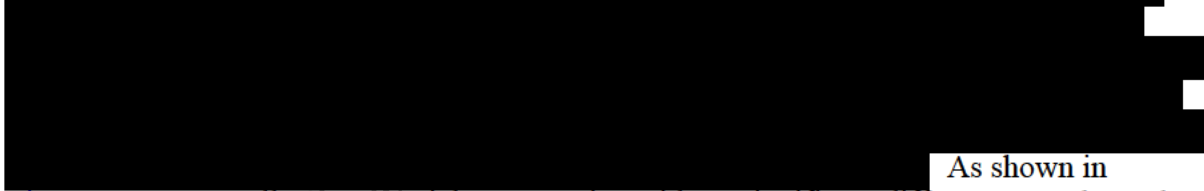
As shown by shallow angle X-ray scattering (SAXS) the vialled, frozen drug product material produced by the classical and upscale processes display similar features 4.2 1st ind.



Figure 3.2.P.2.3-7. Scattering Curves from Vialled/Thawed Drug Product Produced Using Classical Process and Upscale Process



The potency of the drug product produced using the classical and upscale processes was determined using a mouse immunogenicity model. 4.2 1st ind.



As shown in

Figure 3.2.P.2.3-8 all LNP materials were active with no significant differences as shown by the S1-ELISA and the RBD-ELISA.

Figure 3.2.P.2.3-8. Biological Activity of Classical Process and Upscale Process Drug Product in a Mouse Immunogenicity Model

4.2 1st ind.

3.2.P.2.3.6.4. Upscale LNP Manufacturing Process Adjustments for Increased Batch Size

As an initial demonstration of comparability of drug product containing BNT162b2 with LNP production using the Upscale process, results of release testing are presented in [Section 3.2.P.5.4 Batch Analyses](#) for drug product lots EE8492, EE8493, EJ0553, EJ1685, EJ1686 and EK1768 demonstrating no significant differences from previous lots made by the Classical LNP production process. Drug product lots EE8492 and EE8493 were manufactured using the Upscale process with 20 g RNA input. EJ0553, EJ1685, EJ1686 and EK1768 were manufactured utilizing an input of 40 g of RNA and two parallel T-mixers for production of LNPs, effectively doubling the capacity of the LNP fabrication process. Parameters for production of EJ0553, EJ1685, EJ1686 and EK1768 are the same as shown in [Table 3.2.P.2.3-26](#) (Upscale process column), except that two T-mixers are used in parallel to match the increase in RNA input. Results of release testing presented in [Section 3.2.P.5.4 Batch Analyses](#) further demonstrate consistent product quality among lots EE8492, EE8493, EJ0553, EJ1685, EJ1686 and EK1768, supporting the consistency of the Upscale process and further demonstrating the capability of the scale out approach of additional T-mixers for added process capacity and throughput. A comprehensive evaluation of product quality comparability of DP lots from the Upscale process, including the scale out approach with additional T-mixer, and previous Classical process lots is presented in [Section 3.2.P.2.3 Development History](#). For commercial manufacture, the scale out approach will be used to

further increase capacity of the LNP fabrication process with potential for up to 8 T-mixers in parallel.

3.2.P.2.3.6.5. Conclusions

Evaluation of quality attributes of the drug product, including biological activity, indicated that there was no difference in drug product produced from the classical process and the upscale process.

Addition of T-mixers in parallel can be used to increase capacity of the LNP fabrication process for increased batch size.

The process parameters for formation and stabilization of lipid nanoparticles is summarized in Table 3.2.P.2.3-27.

Table 3.2.P.2.3-27. Process Parameters for Formation and Stabilization of LNPs

Process Parameter	Acceptable Range
4.2 1st ind.	4.2 1st ind.

a. Target set-point during LNP formation
Abbreviation: LNP = lipid nanoparticle

3.2.P.2.3.7. Buffer Exchange and Concentration

3.2.P.2.3.7.1. General Description

After fabrication of the LNPs, tangential flow filtration (TFF) is used to perform the buffer exchange and concentration and formulation of the final drug product in the PBS formulation buffer. The formulated LNPs are filtered with a bioburden reduction filter into a holding vessel for final formulation.

3.2.P.2.3.7.2. Development of the Process for Buffer Exchange and Concentration

As described in [Section 3.2.P.2.3.6.3](#), the upscale process was developed to enable larger scale manufacturing for emergency supply and commercial supply. Equipment and materials have been scaled up appropriately at the manufacturing sites to meet the projected batch sizes. [Table 3.2.P.2.3-28](#) shows the parameters used during tangential flow filtration (TFF) to perform the buffer exchange and concentration and the comparison between the classical and upscale processes. After this step the classical and upscale processes converge and there is no difference in the further processing of the LNPs. The total volume of the formulated bulk drug product and the number of vials are increased in the upscale process.

Table 3.2.P.2.3-28. Difference between Classical and Upscale Processes for Buffer Exchange and Concentration

Process Step	Parameter	Classical Process	Upscale Process	Change
TFF1 Buffer Exchange and Concentration	TFF Loading (g/m ²)			
	LNP concentration (mg/mL)			
	EtOH concentration (%)			
	DF volumes			
	Process time (h)			
	Process temperature			
	Final LNP concentration (mg/mL)			
	Membrane material of construction			

Abbreviations: TFF = tangential flow filtration; LNP = lipid nanoparticle; DF = diafiltration; mPES = modified polyethersulfone

3.2.P.2.3.7.3. Development of Buffer Exchange and Concentration Step for Increased Batch Size



Table 3.2.P.2.3-29. In-process Analysis of the TFF Permeate Stream

Process step	pH	Osmolality (mOsmol/kg)
Beginning of DF1	4.2 1st ind.	
Middle of DF1		
End of DF1		
End of C1		
Beginning of DF2		
Middle of DF2		
End of DF2		

Abbreviations: DF = diafiltration; C = concentration

4.2 1st ind.

samples were taken and analyzed to assess product quality at the end of the TFF step (Table 3.2.P.2.3-30). Analytical results met the acceptance criteria demonstrating the effectiveness of the TFF process.

Table 3.2.P.2.3-30. Analysis of the Bulk Drug Product at the End of the TFF Process

Method	Procedure	Limits	Result
RNA encapsulation	Fluorescence assay (Ribogreen)	4.2 1st ind.	
RNA content	Fluorescence assay (Ribogreen)		
LNP size	Dynamic Light Scattering (DLS)		
LNP polydispersity	Dynamic Light Scattering (DLS)		
ALC-0315 content	HPLC-CAD		
ALC-0159 content	HPLC-CAD		
DSPC content	HPLC-CAD		
Cholesterol content	HPLC-CAD		
pH	Potentiometry		
Osmolality	Osmometry		

The resulting bulk drug product was filled on the Vaccine Cell 2 (VC2) filling line at Puurs (engineering lot EK2808). Quality attributes of the final drug product were compared to a representative drug product lot executed at Puurs with Polymun-produced LNPs at approximately 80 L scale (drug product lot EK1768 as shown in [Section 3.2.P.5.4 Batch Analyses](#)). The results of analytical testing for both lots were comparable (Table 3.2.P.2.3-31).

Table 3.2.P.2.3-31. Comparison of Commercial Process with Two TFF Membranes in Series (Engineering Lot EK2808) to Drug Product Lot EK1768

Quality Attribute	Acceptance Criteria	EK1768	EK2808
Appearance	White to off-white suspension	White to off-white suspension	White to off-white suspension
Appearance (visible particulates)	EFVP	EFVP	EFVP
Subvisible particles	Particles $\geq 10 \mu\text{m}$: ≤ 6000 per container	$\geq 10 \mu\text{m}$: 233	$\geq 10 \mu\text{m}$: 113
	Particles $\geq 25 \mu\text{m}$: ≤ 600 per container	$\geq 25 \mu\text{m}$: 10	$\geq 25 \mu\text{m}$: 0
pH	7.4 ± 0.5	7.2	7.2
Osmolality	4.2 1st ind.		
LNP size			
LNP polydispersity			
RNA encapsulation			
RNA content			
ALC-0315 content			
ALC-0159 content			
DSPC content			
Cholesterol content			
In-vitro expression			
RNA integrity			

Abbreviations: EFVP = Essentially free from visible particulates; N/A = data not available at time of filing; PPC = Particles per container

3.2.P.2.3.7.4. Conclusions

Based on lab scale studies and clinical manufacturing studies performed through classical process development and upscale process development, the parameters for the commercial process were determined and are summarized in Table 3.2.P.2.3-32 and Table 3.2.P.2.3-33.

Additionally, quality attributes of the drug product from a 4.2 1st ind. process using a product path 4.2 1st ind. were comparable to those of a representative upscale batch. This confirms the feasibility of the increased batch size process 4.2 1st ind. and supports the manufacturing plan.

Table 3.2.P.2.3-32. Filter Properties for Buffer Exchange and Concentration

Membrane material of construction	4.2 1st ind.
-----------------------------------	--------------

Abbreviations: mPES = modified polyethersulfone

Table 3.2.P.2.3-33. Process Parameters for Buffer Exchange and Concentration

Process Parameter	Acceptable Range
4.2 1st ind.	4.2 1st ind.

a. Target set point, maximum 4.2 1st ind.

Abbreviations: LPM = liters per minute; PBS = phosphate-buffered saline

3.2.P.2.3.8. Concentration Adjustment and Addition of Cryoprotectant

3.2.P.2.3.8.1. General Description

This process step includes mixing of the LNPs after addition of the sucrose cryoprotectant to achieve the final formulated drug product. Upon completion of the TFF and bioburden reduction filtration, the LNPs and the appropriate amount of PBS formulation buffer and 1.2 M sucrose are added to the holding vessel and mixed to achieve the target drug product RNA concentration of 0.5 mg/mL and 300 mM sucrose in PBS buffer.

3.2.P.2.3.8.2. Development of the Process for Concentration Adjustment and Addition of Cryoprotectant

4.2 1st ind.

Studies have been performed in the development laboratory to support the initial/commercial process with additional studies as needed performed during qualification and validation.

3.2.P.2.3.8.3. Conclusions

Based on evaluation of available information and experience during CTM manufacture, the process parameters for concentration adjustment and addition of cryoprotectant are summarized in Table 3.2.P.2.3-34.

Table 3.2.P.2.3-34. Process Parameters for Concentration Adjustment and Addition of Cryoprotectant

Process Parameter	Acceptable Range
4.2 1st ind.	4.2 1st ind.

a. Mixing time and mixing speed will be established based on the results of process qualification studies.

Abbreviation: PBS = phosphate-buffered saline

3.2.P.2.3.9. Comparison Between CTM and Emergency/Commercial Manufacturing Process

The comparison between the CTM and emergency/commercial processes are shown in Table 3.2.P.2.3-35.

Table 3.2.P.2.3-35. Comparison between the Drug Product Process for CTM and Emergency^a/Commercial Supply

Process Step	CTM	Emergency/Commercial Supply
Drug Substance (RNA Batch Size)	4.2 1st ind	
Dilution of Drug Substance (Aqueous Phase)		
Preparation of Organic Phase (Organic Phase)		
LNP Formation and Stabilization (Flow Rates)		
Concentration, Buffer Exchange and Filtration (Filter Surface Area)		
Concentration Adjustment and Addition of Cryoprotectant		
Sterile Filtration (Filter Area)		

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

b. **4.2 1st ind**

Abbreviations: CTM = clinical trial material; LNP = lipid nanoparticle; TFF = tangential flow filtration; PBS = phosphate-buffered saline; NLT = Not less than

3.2.P.2.3.10. Sterile Filtration

3.2.P.2.3.10.1. General Description

4.2 1st ind

4.2 1st ind

3.2.P.2.3.10.2. Process Characterization Studies

3.2.P.2.3.10.2.1. Prior Knowledge

According to the information from the filter manufacturer, sterilizing-grade 0.2 μm hydrophilic PES membranes provide sterility assurance, high flow rates and throughputs. The PES membrane contributes to clean processes due to low extractables, broad chemical compatibility, and non-fiber releasing properties. Filters with hydrophilic PES membranes are designed, developed, and manufactured in accordance with a Quality Management System approved by an accredited registering body to an ISO 9000 Quality Systems Standard and are shipped with a Certificate of Quality. Each cartridge and capsule filter is integrity tested during manufacturing and is supported by a validation ([Section 3.2.P.3.5 Sterilizing Filter Membrane Validation](#)) for compliance with regulatory requirements.

PES filter membrane (0.2 μm) is a commonly used sterilizing grade filter for sterile drug product manufacture in the pharmaceutical and biotechnology industry. In addition, no significant adsorption of LNPs is expected based on manufacturing experience during clinical development. Therefore, this PES filter was chosen to be used in the sterile filtration of the BNT162b2 drug product at commercial scale.

3.2.P.2.3.10.2.2. Filter Sizing

A laboratory scale study was conducted to determine filter capacity (V_{max}) using a 0.2 μm scale down disk with surface area. The filtration pressure target used for commercial manufacturing is with a range of. The laboratory scale V_{max} test was executed using the target pressure of.

The minimum required filter surface area, A_{min} , is estimated based on V_{max} 80% according to the gradual pore-plugging model¹. Filter sizing (A_{min}) for the full scale GMP manufacture of a batch volume is summarized in [Table 3.2.P.2.3-36](#).

Table 3.2.P.2.3-36. Minimum Filter Surface Area Based on Vmax 80%

Study	Lab Scale Filter Size (m ²)	Vmax 80% (L/m ²)	Commercial Process Minimum Area (m ²) Vmax based	Commercial Batch Size (L)
Laboratory scale	4.2 1st ind			

a. Rounded to the nearest tenth

According to the laboratory scale Vmax data, the 4.2 1st ind PES filters for the redundant sterile filtration proposed for commercial manufacturing are appropriately sized based on flow rate and filter capacity, as both filters have a larger surface area than the minimum predicted by the lab-scale study.

3.2.P.2.3.10.2.3. Filtration Shear Stress

The shear rate experienced by BNT162b2 bulk drug product inside filter membrane pores was also evaluated using the Vmax data and the following equation:

4.2 1st ind

Under actual manufacturing conditions the target filtration pressure is 4.2 1st ind. These experimental conditions at 4.2 1st ind show that BNT162b2 bulk drug product is not sensitive to filtration shear stress under targeted manufacturing process conditions.

3.2.P.2.3.10.2.4. Filter Adsorption

Evaluation of adsorption of BNT162b2 drug product to the 0.2 µm sterilizing grade PES filter membranes used in the drug product manufacturing process was performed in the development laboratory. An 0.2 µm, 4.2 1st ind, PES disk filter with effective filtration area of 4.2 1st ind was used for the lab-scale study. 4.2 1st ind

The

filter for use on the commercial lines at Kalamazoo and Puurs is a 4.2 1st ind diameter, 4.2 1st ind PES filter capsule or cartridge. For a 4.2 1st ind batch size, the loss is projected to be 4.2 1st ind. At 0.5 mg/mL, this is a loss of 4.2 1st ind. After completion of filtration, the concentration of the filtrate including the projected loss is 4.2 1st ind. This is not considered a significant loss of BNT162b2 drug product.

Table 3.2.P.2.3-37. Summary of BNT162b2 Filter Adsorption Study

Fraction Number	Fraction Volume (mL)	Total Volume (mL)	RNA concentration (µg/mL)	Encapsulation Efficiency (%)
4.2 1st ind				

Abbreviations: NA = Not applicable

3.2.P.2.3.10.2.5. Filter Flush for Leachables Removal

A dynamic filter flush study was performed by 4.2 1st ind to determine the amount of water needed to flush the 4.2 1st ind PES filter to reduce Total Organic Carbon (TOC) levels measured in the filter effluent to below 0.500 ppm. The threshold of 0.500 ppm is set based on the requirements set forth in USP <643>. The study was performed by the manufacturer with 4.2 1st ind and results of the study indicated that 4.2 1st ind of water flush would reduce organic leachables to below this threshold. Water for Injection (WFI) and/or PBS buffer is used for flushing the filters. As PBS is a simple aqueous solution, it is expected to perform similarly to water.

The BNT162b2 commercial scale process uses 4.2 1st ind PES filters for sterile filtration. The filters used in the vendor study contain identical materials of construction and differ only in surface area, having one-half the area. Since scale up is linear, not less than 4.2 1st ind of WFI or PBS buffer per filter is required for leachables removal. Based on this information the flush volume for the filters in the BNT162b2 manufacturing process has been set to 4.2 1st ind.

3.2.P.2.3.10.2.6. Re-Filtration Process

Laboratory scale re-filtration studies were performed to demonstrate the feasibility of re-filtration of bulk drug product during the sterile filtration step of the commercial drug product manufacturing process. Re-filtration of bulk drug product may be necessary for reasons including, but not limited to, issues related to filter integrity and the integrity of the holding vessel post-sterile filtration. A multiple filtration study was conducted to evaluate the effect of filtration shear stress during drug product manufacture on product quality. A volume of 4.2 1st ind of BNT162b2 development drug product was 4.2 1st ind, each filtration

through a new filter. Samples were analyzed for RNA concentration (µg/mL) and encapsulation efficiency (%) by fluorescence assay, and LNP size (nm) and polydispersity by dynamic light scattering. Results are shown in Table 3.2.P.2.3-38.

Analytical results for RNA concentration, encapsulation efficiency, polydispersity and size of the LNP indicate that quality of the drug product is not adversely affected by **[REDACTED]** filtrations as all samples meet the acceptance criteria. These results support the re-filtration process if needed.

Table 3.2.P.2.3-38. Analytical Test Results for Multiple Filtration Study

Sample (filtrations)	RNA Concentration	Encapsulation Efficiency	Size	Polydispersity
Acceptance Criteria	4.2 1st ind	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]

Abbreviations: MF = multiple filtration

3.2.P.2.3.10.2.7. Filter Validation

Sterile filtration membrane validation studies were executed by **[REDACTED]** for the BNT162b2 initial/commercial drug product manufacturing process and are detailed in [Section 3.2.P.3.5 Sterilizing Filter Membrane Validation](#). The studies for microbial retention, filter compatibility, extractables, and product-specific bubble point testing have shown that the filter device containing a PES 0.2 µm membrane is appropriate for the sterile filtration of final formulated BNT162b2 bulk drug product.

3.2.P.2.3.10.3. Conclusions

During the characterization of the filtration unit operation at laboratory scale, fluid characteristics of the filter, including determination of filter sizing (Vmax) and the impact of filtration shear stress, were assessed. In addition, the BNT162b2 bulk drug product compatibility with the filter including adsorption, and re-filtration was also evaluated. Along with the enhanced prior knowledge and product understanding, clinical manufacturing experience established the foundation for the process executed at full-scale.

3.2.P.2.3.11. Aseptic Filling


3.2.P.2.3.11.1. Fill Weight

The BNT162b2 drug product is a multi-dose vial containing 225 µg/vial with a nominal fill volume of 0.45 mL sterile drug product in a 2 mL vial. Vials are filled to the target fill weight **[REDACTED]**, and a fill weight check is performed at defined time intervals. **[REDACTED]**

[REDACTED] As data are not yet available for the engineering and process performance qualification runs at

the commercial sites, initial line trials conducted on representative lines demonstrate, and provide assurance, that target fill weights are likely to be achieved on the commercial lines on a global basis in advance of qualification data for each and every line. Although not all facilities may be registered in all markets, information from development work performed at multiple facilities is presented as relevant to overall process and product understanding.

4.2 1st ind



4.2 1st ind

4.2 1st ind

In addition to these trials on the commercial lines, fill weights for clinical batch EE8492 filled at Puurs on line WSL5 are shown in [Figure 3.2.P.2.3-11](#). This demonstrates the capability of meeting fill weight specifications during manufacture of a clinical lot using BNT162b2 and the same process design that will be used for commercial manufacturing.

4.2 1st ind

3.2.P.2.3.11.2. Filling Needle Shear Stress

4.2 1st ind

4.2 1st ind.

Table 3.2.P.2.3-39. Vial Fill Volume, Line Speed and Needle Size at Kalamazoo (Placebo) and Puurs (BNT162b2) for Shear Rate Calculation

Factors for Shear Rate Calculation	Conversions
------------------------------------	-------------

4.2 1st ind

4.2 1st ind

3.2.P.2.3.12. Stoppering, Sealing and Capping Vials

Upon the completion of filling, the vials are fully stoppered and capped. After stoppering and capping are complete, drug product vials are visually inspected prior to labelling, freezing and storage at -90 to -60 °C.

An initial study was performed to determine the correlation of the residual seal force (RSF) and container closure integrity (CCI) at -80 °C as a function of a vial-stopper combination and stopper compression. Testing was performed to assess the impact of capping (crimping) force on the ability of the container closure system to maintain integrity. Based on the study results, an Initial Residual Seal Force (Initial RSF) alert limit of 4.2 1st ind. is recommended for setup and monitoring of the BNT162b2 drug product container closure system capping process at commercial manufacturing sites. The RSF test method was developed as a monitoring tool to assess capping setting performance and is expected to provide measurable indication of the consistency of the force applied and maintained by the crimping process in addition to the current manufacturing controls. Testing is summarized in [Section 3.2.P.2.4 Container Closure System](#).

3.2.P.2.3.13. Visual Inspection

100% visual inspection is performed for commercial drug product manufacture. Commercial sites will use automated visual inspection with the ability for manual inspection if needed.

3.2.P.2.3.14. Freezing of Drug Product

After each vial is filled, stoppered, capped and inspected, the labeling operation is performed. Labeled vials are packaged in plastic corrugated trays closed with a lid and moved to the freezing area for freezing prior to storage. Tray configurations are designed to contain either 4.2 1st ind. Freezing results in the stabilization of the vaccine drug product such that a commercially viable shelf life can be achieved. The objective of the freezing process is to ensure complete freezing of the drug product prior to storage.

Freezing rates and hold temperatures affect the structural characteristics of the frozen matrix. Freezing process development as elaborated in this section includes thermal analysis of the BNT162b2 formulation and freezing cycle development.

3.2.P.2.3.14.1. Thermal Analysis of the BNT162b2 Formulation using Modulated Differential Scanning Calorimetry (mDSC)

As described in [Section 3.2.P.2.2 Drug Product](#), differential scanning calorimetry was performed on the BNT162b2 frozen drug product. The drug product exhibits two glass

transition events characteristic of saccharide-containing formulations with onset temperatures at 4.2 1st ind. [REDACTED] The higher temperature event, Tg', is identified as the glass transition of the maximally freeze-concentrated solution. Molecular mobility decreases below the glass transition which prevents instability over time. Based on an understanding of the glassy state dynamics and available stability data for both the BNT162b1 and BNT162b2 drug products stored at -70±10 °C as shown in Section 3.2.P.2.2 Drug Product, instability at temperatures below 4.2 1st ind. [REDACTED] is not expected.

3.2.P.2.3.14.2. Freezing Rate Development

Based on studies previously performed at Puurs, it was estimated that cooling rates

4.2 1st ind. [REDACTED]

4.2 1st ind. [REDACTED]

Different study designs as shown in Table 3.2.P.2.3-40 were used in the development laboratory to evaluate the range of cooling rates encountered in the Puurs experiments and examine even slower cooling rates.

Table 3.2.P.2.3-40. Cooling Rates Examined in Development Studies


4.2 1st ind. [REDACTED]

4.2 1st ind. [REDACTED]

3.2.P.2.3.14.2.1. Controlled (Programmed) Rate Freezing

Initial experiments were performed using a freeze-dryer to assess the relationship between programmed and actual product cooling rates. 4.2 1st ind. [REDACTED]

4.2 1st ind.



4.2 1st ind.



4.2 1st ind.




The next study was performed with BNT162b2 drug product (4.2 1st ind. Lot FM-1192A) formulated in 4.2 1st ind. . Boxes containing drug product vials were placed on the tray of a freeze-dryer as shown in Figure 3.2.P.2.3-13.

Figure 3.2.P.2.3-13. Placement of Boxes Containing Vials within a Freeze-dryer Tray

4.2 1st ind.



4.2 1st ind.



4.2 1st ind.

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

4.2 1st ind.

[REDACTED]

[REDACTED]

3.2.P.2.3.14.2.2. Non-programmed Freeze

3.2.P.2.3.14.2.2.1. Freeze in -80 °C Freezer

This study used the same study design for drug product vial preparation and placement in the freezer (see [Figure 3.2.P.2.3-13](#)) but the BNT162b2 (4.2 1st ind. Lot FM-1192A) drug product vials were frozen on the shelf of a -80 °C freezer.

4.2 1st ind.

[REDACTED]

3.2.P.2.3.14.2.3. Freeze in Liquid Nitrogen Vapor or Liquid Nitrogen

Immersion in liquid nitrogen or suspension in the vapor phase of liquid nitrogen is expected to yield the fastest cooling. BNT162b2 (4.2 1st ind. Lot FM-1192A) drug product vials were frozen by suspending the vials in the vapor phase or by immersion in liquid nitrogen to assess the impact of the faster cooling rates on product quality attributes. 4.2 1st ind.

[REDACTED]

[REDACTED]

Vials were later removed from the freezer, packaged as described above, and shipped for analysis.

As shown in Table 3.2.P.2.3-41 4.2 1st ind.

[REDACTED]

The summary of cooling rates at each investigated condition is shown in Table 3.2.P.2.3-41.

Table 3.2.P.2.3-41. Summary of Cooling Rates Calculated as Averages Based on Thermocouple Data

4.2 1st ind.

3.2.P.2.3.14.2.4. Impact of Cooling/Freezing Rates on Product Quality at 0.5 mg/mL

Samples from the experiments were evaluated for LNP size and polydispersity by dynamic light scattering, % encapsulation by fluorescence assay, in vitro expression by cell-based flow cytometry assay, and RNA integrity and Poly(A) by ddPCR. Results of the evaluation are shown in Table 3.2.P.2.3-42 and [Figure 3.2.P.2.3-14](#), [Figure 3.2.P.2.3-15](#), [Figure 3.2.P.2.3-16](#) and [Figure 3.2.P.2.3-17](#) for 0.5 mg/mL samples. There were no trends as a function of cooling rates for any of the assay results.

Table 3.2.P.2.3-42. Effect of Cooling Rates on BNT162b2 Drug Product Quality Attributes



Abbreviations: PDI = polydispersity index; NT = not tested; LN₂ = liquid nitrogen

4.2 1st ind.

4.2 1st ind.

4.2 1st ind.

4.2 1st ind.

3.2.P.2.3.14.2.5. Impact of Extra Slow Cooling Rate (0.02 °C/min) on BNT162b2 and BNT162b1 Drug Product

Additional experiments were performed to assess the impact of an extra slow cooling rate on both BNT162b2 and BNT162b1 drug product. In this bridging study, BNT162b2 and BNT162b1 drug product were subjected to cooling/freezing at the extra slow rate of 0.02 °C/minute. 4.2 1st ind. [REDACTED]

4.2 1st ind. [REDACTED]

[REDACTED]

4.2 1st ind.

4.2 1st ind.

4.2 1st ind.

Abbreviations: NT = Not tested

3.2.P.2.3.14.2.6. Conclusions

4.2 1st ind.

These studies support the commercial scale process parameters for freezing as shown in Table 3.2.P.2.3-44. Additionally, other packaging configurations may be used if development studies confirm these conclusions.

Table 3.2.P.2.3-44. Process Parameters for Freezing

Process Parameter	Acceptable Range
Freezing temperature	-90 to -60 °C

3.2.P.2.3.15. Drug Product Robustness to Freezing and Warming During Storage

For long term storage, the BNT162b2 drug product is frozen to target -70 °C with long term storage at -90 °C to -60 °C. During the freezing and frozen storage operations the drug product will likely be exposed to room temperature several times. A study was performed using freezing and warming cycling to determine the effect of potential temperature excursions on the stability of the drug product. 4.2 1st ind.

[REDACTED]

- 4.2 1st ind.

[REDACTED]

[REDACTED]

[REDACTED]

4.2 1st ind.

[REDACTED]

4.2 1st ind.

4.2 1st ind.

4.2 1st ind.

Figure 3.2.P.2.3-20. Size and Polydispersity of Drug Product at Terminal Sampling Points for Each Process



Figure 3.2.P.2.3-21. Encapsulation Efficiency (%) and Total mRNA Content (mg/mL) at Terminal Sampling Points for Each Process



4.2 1st ind.

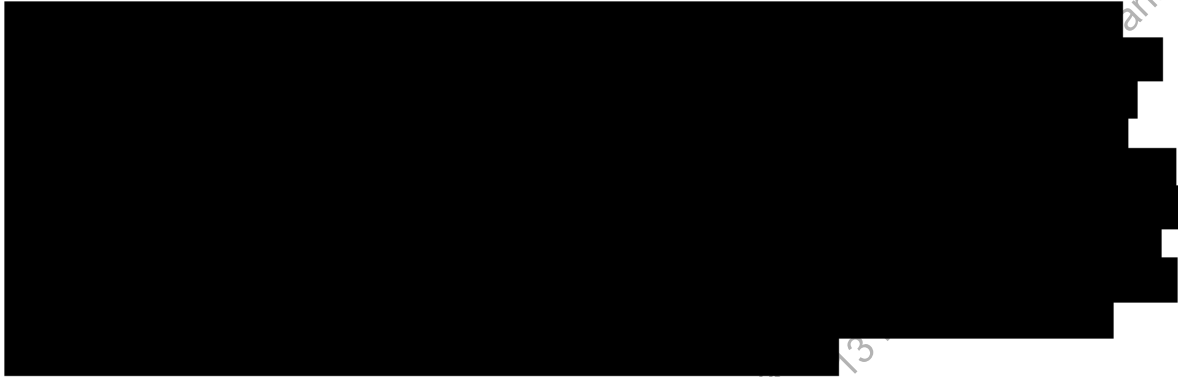


Table 3.2.P.2.3-46. Average Temperature and Cumulative Residence Time for Isothermal Holds during Cycling in the Standard Process, Back up Process and Flexible Process

4.2 1st ind.

Abbreviations: NA = not applicable; Tp = product temperature

The results of this study support the time out of freezing ranges for drug product during freezer transfers and preparation for shipping and for freezing/warming post freezing step during manufacture and packaging as shown in [Table 3.2.P.2.3-47](#).

Table 3.2.P.2.3-47. Time Out of Storage Condition for BNT162b2 Drug Product

4.2 1st ind.

Abbreviations: CPP = critical process parameter

3.2.P.2.3.15.1. Conclusions

Results of the freezing/warming cycling study support the time out of -90 to -60 °C as defined in the manufacturing process in Section 3.2.P.3.4 Process Hold Times – Fill and Finish.

3.2.P.2.3.16. Drug Product Storage

Drug product vials are stored frozen at -90 to -60 °C. Stability data for the BNT162b2 drug product is provided in Section 3.2.P.8.3 Stability Data.

3.2.P.2.3.17. Drug Product Shipping

Shipping validation studies for the frozen drug product have also been performed and are described in [Section 3.2.P.3.5 Shipping Validation](#).

- 1 [Badmington F, Honig E, Payne M, Wilkins R. Vmax testing for practical microfiltration train scale-up in biopharmaceutical processing. Pharm Technol 1995; 19: 64-76](#)
- 2 [Zhao P, Hou X, Yan J, Du S, Xue Y, Li W, Xiang G, and Dong Y. Long-term storage of lipid-like nanoparticles for mRNA delivery, Bioact. Mater. 2020, 5:2, 358-363.](#)
- 3 [Ball, R. L., Bajaj, P., Whitehead, K. A. Achieving long-term stability of lipid nanoparticles: examining the effect of pH, temperature and lyophilization. Int. J. Nanomedicine. 2016. 12, 305-315.](#)
- 4 [Kasper, J.C., Pikal, M.J., Friess, W. Investigations on polyplex stability during the freezing step of lyophilization using controlled ice nucleation – the importance of residence time in the low-viscosity fluid state. J. Pharm. Sci. 2013. 102, 929-946.](#)