

## TABLE OF CONTENTS

LIST OF TABLES.....	4
LIST OF FIGURES .....	7
3.2.P.2.3. PROCESS DEVELOPMENT AND CHARACTERIZATION .....	9
3.2.P.2.3.1. Process Development and Characterization Studies for Unit Operations.....	9
3.2.P.2.3.2. Hold Times.....	15
3.2.P.2.3.2.1. Stability of Lipids Solubilized in Ethanol .....	15
3.2.P.2.3.2.1.1. Conclusion .....	17
3.2.P.2.3.2.2. Lipid Nanoparticle In-process Holds.....	17
3.2.P.2.3.2.2.1. Conclusion .....	19
3.2.P.2.3.2.3. Laboratory Scale Product Contact Materials Compatibility and Light Exposure Studies .....	19
3.2.P.2.3.2.3.1. Product Contact Materials Compatibility .....	19
3.2.P.2.3.2.3.2. Light Exposure Study.....	19
3.2.P.2.3.2.4. Drug Product Hold Times for Packing, Shipping and Point of Use .....	26
3.2.P.2.3.2.4.1. Conclusions.....	28
3.2.P.2.3.2.5. Information from Formal Stability Studies .....	28
3.2.P.2.3.2.6. Full-Scale Manufacturing Experience .....	28
3.2.P.2.3.3. Drug Substance Thaw .....	28
3.2.P.2.3.3.1. General Description.....	28
3.2.P.2.3.3.2. Characterization of Drug Substance Freezing and Thawing Operations .....	29
3.2.P.2.3.3.3. Conclusions .....	32
3.2.P.2.3.4. Dilution and Mixing of Drug Substance .....	32
3.2.P.2.3.4.1. General Description.....	32
3.2.P.2.3.4.2. Mixing for Dilution of Drug Substance.....	32
3.2.P.2.3.4.3. Conclusions .....	33
3.2.P.2.3.5. Preparation of the Organic Phase.....	33
3.2.P.2.3.5.1. General Description.....	33
3.2.P.2.3.5.2. Development of the Organic Phase Preparation Process .....	33
3.2.P.2.3.5.3. Conclusions .....	33

3.2.P.2.3.6. Lipid Nanoparticle (LNP) Formation and Stabilization .....	34
3.2.P.2.3.6.1. General Description.....	34
3.2.P.2.3.6.2. Development of the LNP Manufacturing Research Scale Process .....	35
3.2.P.2.3.6.3. Development of LNP Manufacturing “Classical Process” and “Upscale Process” .....	35
3.2.P.2.3.6.4. Upscale LNP Manufacturing Process Adjustments for Increased Batch Size .....	41
3.2.P.2.3.6.5. Conclusions .....	42
3.2.P.2.3.7. Buffer Exchange and Concentration .....	42
3.2.P.2.3.7.1. General Description.....	42
3.2.P.2.3.7.2. Development of the Process for Buffer Exchange and Concentration.....	42
3.2.P.2.3.7.3. Development of Buffer Exchange and Concentration Step for Increased Batch Size .....	43
3.2.P.2.3.7.4. Conclusions .....	45
3.2.P.2.3.8. Concentration Adjustment and Addition of Cryoprotectant .....	46
3.2.P.2.3.8.1. General Description.....	46
3.2.P.2.3.8.2. Development of the Process for Concentration Adjustment and Addition of Cryoprotectant .....	46
3.2.P.2.3.8.3. Conclusions .....	46
3.2.P.2.3.9. Comparison Between CTM and Emergency/Commercial Manufacturing Process .....	47
3.2.P.2.3.10. Sterile Filtration .....	47
3.2.P.2.3.10.1. General Description.....	47
3.2.P.2.3.10.2. Process Characterization Studies.....	48
3.2.P.2.3.10.2.1. Prior Knowledge .....	48
3.2.P.2.3.10.2.2. Filter Sizing.....	48
3.2.P.2.3.10.2.3. Filtration Shear Stress .....	49
3.2.P.2.3.10.2.4. Filter Adsorption .....	49
3.2.P.2.3.10.2.5. Filter Flush for Leachables Removal .....	50
3.2.P.2.3.10.2.6. Re-Filtration Process.....	50
3.2.P.2.3.10.2.7. Filter Validation .....	51
3.2.P.2.3.10.3. Conclusions .....	51

3.2.P.2.3.11. Aseptic Filling.....	51
3.2.P.2.3.11.1. Fill Weight.....	51
3.2.P.2.3.11.2. Filling Needle Shear Stress .....	54
3.2.P.2.3.12. Stoppering, Sealing and Capping Vials .....	55
3.2.P.2.3.13. Visual Inspection.....	55
3.2.P.2.3.14. Freezing of Drug Product.....	55
3.2.P.2.3.14.1. Thermal Analysis of the BNT162b2 Formulation using Modulated Differential Scanning Calorimetry (mDSC).....	55
3.2.P.2.3.14.2. Freezing Rate Development .....	56
3.2.P.2.3.14.2.1. Controlled (Programmed) Rate Freezing .....	56
3.2.P.2.3.14.2.2. Non-programmed Freeze .....	58
3.2.P.2.3.14.2.3. Freeze in Liquid Nitrogen Vapor or Liquid Nitrogen.....	59
3.2.P.2.3.14.2.4. Impact of Cooling/Freezing Rates on Product Quality at 0.5 mg/mL .....	60
3.2.P.2.3.14.2.5. Impact of Extra Slow Cooling Rate (0.02 °C/min) on BNT162b2 and BNT162b1 Drug Product .....	63
3.2.P.2.3.14.2.6. Conclusions.....	65
3.2.P.2.3.15. Drug Product Robustness to Freezing and Warming During Storage .....	66
3.2.P.2.3.15.1. Conclusions .....	70
3.2.P.2.3.16. Drug Product Storage.....	70
3.2.P.2.3.17. Drug Product Shipping.....	70

## LIST OF TABLES

Table 3.2.P.2.3-1. Manufacturing Process Characterization Strategy.....	13
Table 3.2.P.2.3-2. Study Design for Lipids Hold Time Support – Lipid Concentration .....	15
Table 3.2.P.2.3-3. Lipid Integrity in Ethanol at 25 °C up to 96 Hours for 15 mg/mL Mixture (µg/mL) (Experiment 1) .....	16
Table 3.2.P.2.3-4. Lipid Integrity in Ethanol at 25 °C up to 4 Days (96 Hours) for 30 mg/mL Mixture (µg/mL) (Experiment 2).....	16
Table 3.2.P.2.3-5. List of Materials for LNP Fabrication .....	18
Table 3.2.P.2.3-6. LNP Hold Time Study Experimental Design .....	18
Table 3.2.P.2.3-7. Results of Analysis of Control and Final Sample from LNP Hold Time Study.....	19
Table 3.2.P.2.3-8. Acceptable Hold Times during LNP Manufacture.....	19
Table 3.2.P.2.3-9. Kalamazoo and Puurs Facilities Cumulative Light Exposures for Development Study .....	20
Table 3.2.P.2.3-10. Analytical Testing for Light Exposure Study.....	21
Table 3.2.P.2.3-11. Drug Substance Sample Exposure Times and Intensity Measurements .....	21
Table 3.2.P.2.3-12. Drug Product Sample Exposure Times and Intensity Measurements .....	22
Table 3.2.P.2.3-13. Analytical Test Results for Drug Substance Exposed to Visible and UV Light.....	22
Table 3.2.P.2.3-14. Analytical Test Results for Drug Product Exposed to Visible and UV Light.....	23
Table 3.2.P.2.3-15. Results of Drug Product Subvisible Particle Testing .....	24
Table 3.2.P.2.3-16. Suggested Process Times Based on Light Exposure Study.....	25
Table 3.2.P.2.3-17. Study Design for Packing, Shipping and Point of Use Hold Time Support.....	26
Table 3.2.P.2.3-18. BNT162b2 Analytical Assays for Evaluation of Time Out of Condition Study Samples .....	27
Table 3.2.P.2.3-19. Physicochemical Attributes and In Vitro Potency Testing Results for Time Out of Condition Study Samples .....	27
Table 3.2.P.2.3-20. Product Quality of BNT162b1 Drug Substance: Freeze and Thaw Study .....	30
Table 3.2.P.2.3-21. Product Quality of BNT162b2 Drug Substance: Freeze and Thaw Cycling Study .....	31
Table 3.2.P.2.3-22. Process Parameters for Controlled Drug Substance Thaw .....	32

Table 3.2.P.2.3-23. Process Parameters for Controlled Room Temperature Drug Substance Thaw .....	32
Table 3.2.P.2.3-24. Mixing Parameters for Dilution of Drug Substance .....	33
Table 3.2.P.2.3-25. Process Parameters for Preparation of Organic Phase .....	34
Table 3.2.P.2.3-26. Differences between Classical and Upscale Processes .....	36
Table 3.2.P.2.3-27. Process Parameters for Formation and Stabilization of LNPs .....	42
Table 3.2.P.2.3-28. Difference between Classical and Upscale Processes for Buffer Exchange and Concentration .....	43
Table 3.2.P.2.3-29. In-process Analysis of the TFF Permeate Stream .....	44
Table 3.2.P.2.3-30. Analysis of the Bulk Drug Product at the End of the TFF Process .....	44
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 .....	45
Table 3.2.P.2.3-32. Filter Properties for Buffer Exchange and Concentration .....	45
Table 3.2.P.2.3-33. Process Parameters for Buffer Exchange and Concentration .....	46
Table 3.2.P.2.3-34. Process Parameters for Concentration Adjustment and Addition of Cryoprotectant .....	46
Table 3.2.P.2.3-35. Comparison between the Drug Product Process for CTM and Emergency/Commercial Supply .....	47
Table 3.2.P.2.3-36. Minimum Filter Surface Area Based on Vmax 80% .....	49
Table 3.2.P.2.3-37. Summary of BNT162b2 Filter Adsorption Study .....	50
Table 3.2.P.2.3-38. Analytical Test Results for Multiple Filtration Study .....	51
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 .....	54
Table 3.2.P.2.3-40. Cooling Rates Examined in Development Studies .....	56
Table 3.2.P.2.3-41. Summary of Cooling Rates Calculated as Averages Based on Thermocouple Data .....	59
Table 3.2.P.2.3-42. Effect of Cooling Rates on BNT162b2 Drug Product Quality Attributes .....	60
Table 3.2.P.2.3-43. Effect of Extra Slow Cooling Rate on BNT162b2 and BNT162b1 Drug Product .....	65
Table 3.2.P.2.3-44. Process Parameters for Freezing .....	65
Table 3.2.P.2.3-45. Results of Stability Testing of BNT162b1 Drug Product Subjected to Thermal Cycling .....	67
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 .....	69

Table 3.2.P.2.3-47. Time Out of Storage Condition for BNT162b2 Drug Product .....70

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## LIST OF FIGURES

Figure 3.2.P.2.3-1. Lipid Integrity in Ethanol at 25 °C for up to 4 Days (96 hours) for 15 mg/mL Mixture (% of weighed lipid) (Experiment 1) .....	16
Figure 3.2.P.2.3-2. Lipid Integrity in Ethanol at 25 °C for up to 4 Days (96 hours) for 30 mg/mL Mixture (% of weighed lipid) (Experiment 2) .....	17
Figure 3.2.P.2.3-3. Comparability of the Particle Size for LNPs Produced by the Classical Process and Upscale Process.....	37
Figure 3.2.P.2.3-4. Comparability of Polydispersity Index (PDI) for LNPs Produced by the Classical Process and Upscale Process.....	38
Figure 3.2.P.2.3-5. Comparability of Encapsulation Efficiency (%) for LNPs Produced by the Classical Process and Upscale Process.....	38
Figure 3.2.P.2.3-6. Comparability of the N/P Ratio for LNPs Produced by the Classical Process and Upscale Process .....	39
Figure 3.2.P.2.3-7. Scattering Curves from Vialled/Thawed Drug Product Produced Using Classical Process and Upscale Process .....	40
Figure 3.2.P.2.3-8. Biological Activity of Classical Process and Upscale Process Drug Product in a Mouse Immunogenicity Model .....	41
Figure 3.2.P.2.3-9. Fill Weight Capability Using Placebo (Surrogate Nanoparticles) on the Kalamazoo Commercial Line 18 .....	52
Figure 3.2.P.2.3-10. Fill Weight Capability Using Water on the Puurs Commercial Line FC2 .....	53
Figure 3.2.P.2.3-11. Fill Weights for BNT162b2 Drug Product Produced on the WSL5 Fill Line for Clinical Batch EE8492 .....	54
Figure 3.2.P.2.3-12. Placement of Thermocouples and Boxes within a Freeze-dryer Tray.....	57
Figure 3.2.P.2.3-13. Placement of Boxes Containing Vials within a Freeze-dryer Tray.....	57
Figure 3.2.P.2.3-14. Size and Polydispersity of BNT162b2 Drug Product, 0.5 mg/mL, after First Freeze to -70 °C.....	61
Figure 3.2.P.2.3-15. Size and Polydispersity of BNT162b2 Drug Product, 0.5 mg/mL, after Freeze, Warm to -40 °C and Refreeze to -70 °C .....	61
Figure 3.2.P.2.3-16. BNT162b2 Drug Product, 0.5 mg/mL, Encapsulation Efficiency (%) and Concentration after First Freeze to -70 °C .....	62
Figure 3.2.P.2.3-17. BNT162b2 Drug Product, 0.5 mg/mL, Encapsulation Efficiency (%) and Concentration after Freeze, Warm to -40 °C and Refreeze to -70 °C .....	62
Figure 3.2.P.2.3-18. Cooling/Freezing Profiles of the BNT162b1 and BNT162b2 Drug Product Produced in the Development Laboratory .....	64

Figure 3.2.P.2.3-19.	Layout of Drug Product Vials: Drug Product (blue circles), Drug Product with Thermocouple (red circles), and Ethanol (open circles).....	67
Figure 3.2.P.2.3-20.	Size and Polydispersity of Drug Product at Terminal Sampling Points for Each Process .....	68
Figure 3.2.P.2.3-21.	Encapsulation Efficiency (%) and Total mRNA Content (mg/mL) at Terminal Sampling Points for Each Process .....	68



### 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 <sup>a</sup> 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) <sup>a</sup> Potency/Mouse immunogenicity <sup>a</sup> RNA Integrity/CGE RNA Content and RNA Encapsulation/Fluorescence LNP Size and Polydispersity/DLS In Vitro Expression/Cell-based flow cytometry <sup>b</sup>
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 <sup>b</sup> RNA integrity/CGE Poly(A) Tail/ddPCR <sup>b</sup>
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 <sup>b</sup> Potency/Mouse Immunogenicity <sup>a</sup>
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 <sup>b</sup>

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 <sup>a</sup> 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 1st ind					T	T
Final Hold Sample	4.2 1st ind					T	T

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 1st ind	
Time from beginning to completion of TFF with additional hold	4.2 1st ind	
Time from end of previous hold at RT with additional hold	4.2 1st ind	
Batch hold after bioburden reduction filtration at 2-8 °C	4.2 1st ind	
LNPs diluted to target DP with addition of sucrose	4.2 1st ind	

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
<b>Drug Substance</b>							
Thawing	4.2 1st ind						
<b>Drug Product</b>							
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 <sup>2</sup>	mWh/cm <sup>2</sup>
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 <sup>2</sup>	Cumulative mWh/cm <sup>2</sup>

### 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

this result and the exposed samples, the lowest of which was [REDACTED], is attributed to method variability and is not considered to be an effect of light exposure.

The in vitro expression assay was conducted with two sample dilutions: 4.2 1st ind [REDACTED]

[REDACTED] This pattern was attributed to variability of this cell-based assay. These in vitro expression changes were not attributed to light exposure.

**Table 3.2.P.2.3-14. Analytical Test Results for Drug Product Exposed to Visible and UV Light**

Sample	Actual Time (Hours)	Visual Appearance	pH	Dynamic Light Scattering		RNA Integrity (%)	RNA Content (mg/mL)	EE (%)	IVE (%)	
				Size (nm)	PDI				100 ng	150 ng
[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]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]

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

The drug product samples were also tested for subvisible particles as shown in Table 3.2.P.2.3-15. Samples were diluted in PBS, 300 mM sucrose, pH 7.4 at 4.2 1st ind. Results of the testing indicated there was no effect of light exposure on the formation of subvisible particles.

**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 <sup>2</sup> )	KZO		Puurs		Intensity (mW/cm <sup>2</sup> )	Hrs
				Intensity (Lux)	Hours	Intensity (Lux)	Hours		Intensity (mW/cm <sup>2</sup> )	Hrs	Intensity (mW/cm <sup>2</sup> )	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 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				2
Drug Product	4.2 1st ind				3
	4.2 1st ind				4
	Freeze/Thaw Cycling – 4 Cycles				
	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

a. Included in calculation of 4.2 1st ind  
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) <sup>a</sup>	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.