

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. at room temperature was performed before analysis.

b. RNA concentration below target is most likely a result of sampling heterogeneity as the result from 4.2 1st ind. is within the acceptance criteria.

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”

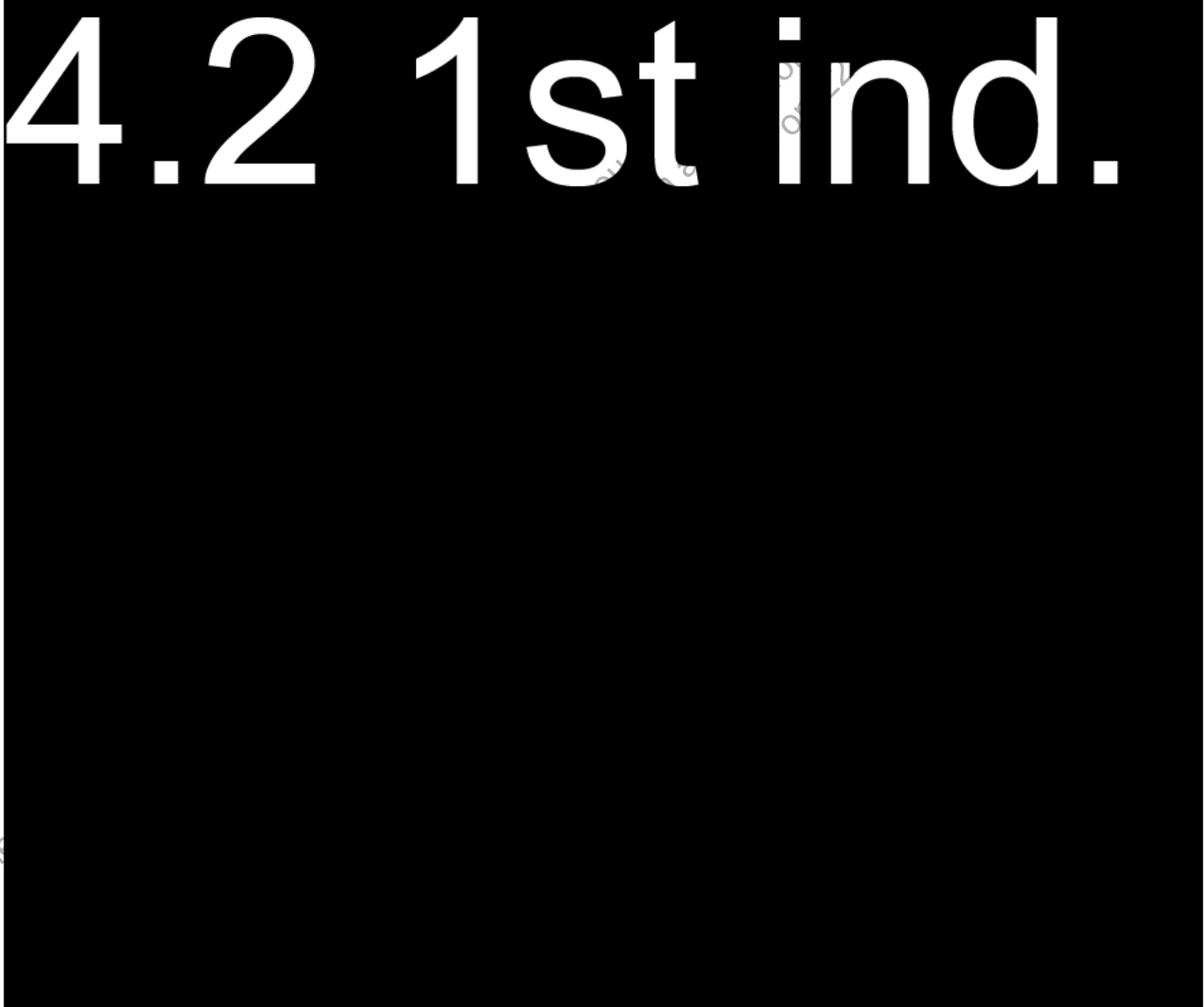
A scaled-up process, termed “Classical Process” has been developed at Acuitas Therapeutics based on the same mixing apparatus configuration and using the same principles as the research scale process described above. Pumps more suitable to higher flow rates were introduced and conventional tangential 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



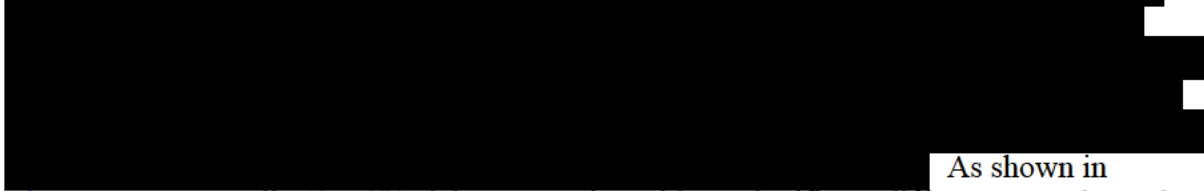
As shown by shallow angle X-ray scattering (SAXS) the vialled, frozen drug product material produced by the classical and upscale processes display similar features 4.2 1st ind.



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