Table of Contents

List of Tables

List of Figures

3.2.P.2.3 Manufacturing Process Development

3.2.P.2.3.1 Introduction

The mRNA-1273 Drug Product manufacturing process is a conventional aseptic process that includes the following basic steps: thawing and pooling of mRNA-1273 LNP, clarification, dilution to 0.20 mg/mL, sterile filtration (redundant 0.2 µm filters), filling into vials, visual inspection, and freezing.

Moderna utilizes part numbers to distinguish different processes and different process scales. A summary of process part numbers is shown in Table 1. A batch genealogy is provided in Table 3.

Table 1: Part Numbers for mRNA-1273 Processes

Manufacturing Scale	CX-024414 mRNA	[redacted]	mRNA-1273 LNP	mRNA-1273 Drug Product
PVU	PN 84100		N/A [a]	PN 85201
Scale up Process (Scale A)	PN 40072		PN 50068	PN 60075 (Moderna) PN 60073 (Catalent) ([redacted] multiple dose vials)
Initial Scale B	PN 40075 (Lonza 20 L IVT)		PN 50073 (100 g mRNA)	N/A
Scale B	PN 40074 (60 L IVT)		PN 50075 (200 g mRNA)	PN 60073 (Ompi vials) PN 60079 (SIO2 vials) ([redacted] multiple dose vials)

Abbreviations: DP = drug product; LNP = lipid nanoparticle; N/A = not applicable; PVU = personalized vaccine unit
a) mRNA-1273 LNP was not included performed as part of the integrated manufacturing process at the PVU scale.

[redacted]

3.2.P.2.3.2 Manufacturing Process Overview

3.2.P.2.3.2.1 PVU Process

The manufacturing process used to produce the Phase 1 and Phase 2 clinical trial material consisted of an integrated manufacturing process (Personal Vaccine Unit [PVU] Process) that incorporated both CX-024414 mRNA and mRNA-1273 lipid nanoparticle (LNP) processes into a single process to produce mRNA-1273 Drug Product (DP) (Figure 1). [redacted] the mRNA-1273 Drug Product release included both mRNA-1273 Drug Product release specifications as well as intermediate (mRNA Process Intermediate [MPI]) in-process specifications. This integrated process used equipment in ModernaTX, Inc.'s small-scale PVU.

[redacted] FPI is the nomenclature adopted for mRNA-1273 LNP in the PVU process. [redacted] the entire small-scale manufacturing process is released with a single specification, with release data from both the MPI and the final mRNA-1273 Drug Product.

3.2.P.2.3.2.2 Scale A

The manufacturing process used to produce Phase 3 clinical trial material utilized scale up (Scale A) of the MPI, [redacted] and FPI processes and utilized equipment from ModernaTX, Inc's clinical scale manufacturing suites. With scale up, the processing was divided into [redacted] distinct DS processes and a separate DP process (Figure 1):

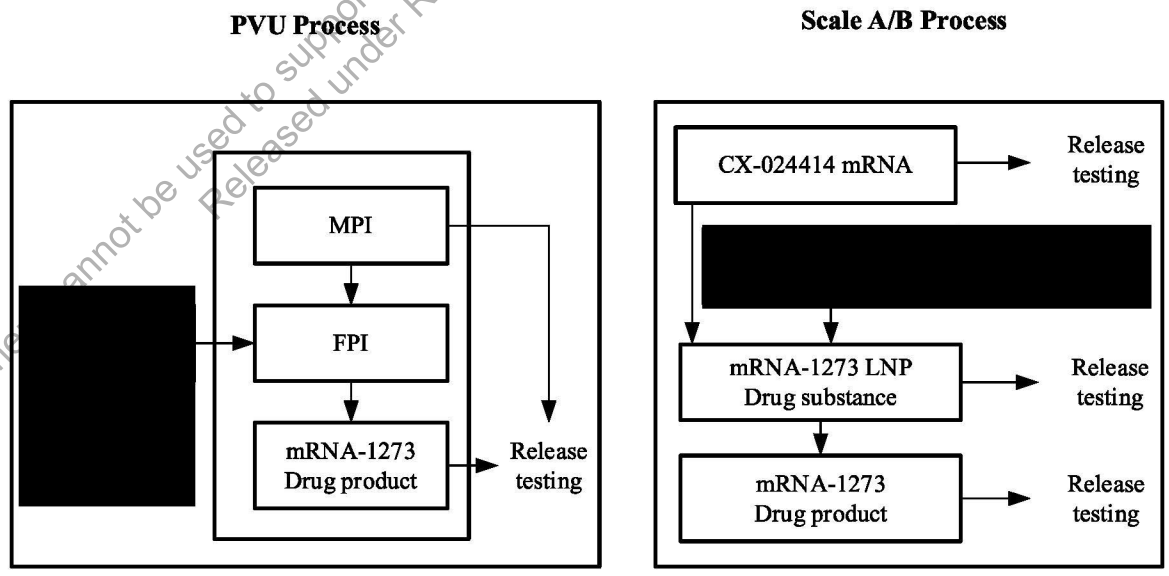
- CX-024414 mRNA manufacturing process (10 L in vitro transcription [IVT] scale and further scale-up at Lonza to 20L IVT scale)

[redacted]

- mRNA-1273 LNP manufacturing process (nominal 15 g mRNA scale)
- mRNA-1273 Drug Product manufacturing process (nominal [redacted] vial scale)

The scale up of the manufacturing processes included an increase in scale compared to the PVU process and changes to equipment to meet demand. The CX-024414 mRNA [redacted] manufacturing process underwent further process enhancements during the manufacture of Phase 3 and process performance qualification (PPQ) material. [redacted] The mRNA-1273 Drug Product process was also transferred to a Contract Manufacturing Organization (CMO) in order to increase scale. Norwood Scale A mRNA-1273 Drug Product was established at a nominal [redacted] vial batch size and transferred to Catalent Biologics, LLC (Bloomington IN) to scale to up to a nominal [redacted] vial batch size.

Figure 1: Overview of Manufacturing mRNA-1273 Processes



Abbreviations: FPI = formulated product intermediate; LNP = lipid nanoparticle; MPI = mRNA product intermediate

3.2.P.2.3.2.3 Scale B

Scale up to Scale B occurred with the same mRNA-1273 processes as established for Scale A (Figure 1). Additional scale up of mRNA-1273 processes is required for commercial supply. Scale-up to Scale B occurred at Norwood as well as additional CMOs. These include Lonza Biologics, Inc (Portsmouth, NH,) and Catalent (Bloomington, IN) to enable commercial manufacturing capabilities. Scale-up activities and site changes will be qualified as part of the process performance qualification. The mRNA-1273 manufacturing process is designed to be scalable and product produced to remain comparable as this process is scaled up.

Scale up to Scale B follows the following strategy:

- CX-024414 mRNA manufacturing process is scaled initially to 20 L IVT scale (Lonza, Portsmouth only) to gain scale up experience. The final Scale B will be 60 L IVT scale at all sites (Norwood, Lonza Portsmouth).

[redacted]

- mRNA-1273 LNP manufacturing process is scaled initially to 100 g mRNA scale to gain scale up experience. The final Scale B is 200 g mRNA scale.
- mRNA-1273 Drug Product manufacturing process is scaled up to 150,000 vial batch size at CMOs. Currently, manufacture is planned at Catalent Biologics, LLC (Bloomington, IN).

A summary of mRNA-1273 Scale B batch scale and manufacturing sites is shown in Table 2.

Table 2: Scale B Manufacturing Scale for mRNA-1273 Processes

Process	Manufacturing Site(s)	Nominal Batch Scale
CX-024414 mRNA	ModernaTX, Inc. (Norwood, MA) Lonza (Portsmouth, NH)	20 – 60 L IVT mRNA [(a)]
[redacted]		
mRNA-1273 LNP	ModernaTX, Inc. (Norwood, MA) Lonza (Portsmouth, NH)	100 – 200 g mRNA [(c)]
mRNA-1273 Drug Product	Catalent (Bloomington, IN)	75,000 - 150,000 multiple dose vials

a) Initial scale at Lonza Portsmouth is 20 L IVT to gain manufacturing experience. The 20 L IVT scale will be part of PPQ activities at Lonza Portsmouth only. The 20L IVT scale will not be performed at Norwood.

[redacted]

c) Initial scale will be at 100 g mRNA to gain manufacturing experience and will be part of PPQ activities.

Detailed information on changes is provided in the following sections.

3.2.P.2.3.3 mRNA-1273 Drug Product Overview

A summary of mRNA-1273 Drug Product batches for clinical use and PPQ lots manufactured to date is provided in Table 3.

Table 3: Batch Genealogy

CX-024414 mRNA Lot/PN	[redacted] Lot/PN	mRNA-1273 LNP Lot/PN	DP Lot/PN [a]	Process/ Scale	Strength (mg/mL)	DOM	Manufacturer	Disposition / Use
8410000101 (PN: 84100)	5005919001 [redacted]	N/A [a]	8520100101 (PN: 85201)	PVU [a] 68 mL	0.5	07 Feb 2020	ModernaTX, Inc. (Norwood, MA)	Phase 1 (DMID Protocol 20-0003)
8410000102 (PN: 84100)	5005919001 [redacted]	N/A [a]	8520100102 (PN: 85201)	PVU [a] 85 mL		19 Mar 2020		Phase 2 (Study P201)
8410000103 (PN: 84100)	5005919001 [redacted]	N/A [a]	8520100103 (PN: 85201)	PVU [a] 85 mL		23 Mar 2020		Phase 2 (Study P201)
8410000104 (PN: 84100)	5005919001 [redacted]	N/A [a]	8520100104 (PN: 85201)	PVU [a] 65 mL		02 Apr 2020		Phase 2 (Study P201)
4007220002 (PN: 40072)	5005920004 [redacted]	5006820002 (PN: 50068)	6007520001 (PN: 60075)	Scale A IVT 10 L	0.20	28 May 2020		Phase 3 (Study P301)
4007220002 (PN: 40072)	5005920004 [redacted]	5006820002 (PN: 50068)	6007520002 (PN: 60075)			02 Jun 2020		Phase 3 (Study P301)
4007220002 (PN: 40072)	5005920004 [redacted]	5006820002 (PN: 50068)	6007520003 (PN: 60075)			04 Jun 2020		Phase 3 (Study P301)
4007220003 (PN: 40072)	5006920001 [redacted]	5006820003 (PN: 50068)	6007520004 (PN: 60075)			25 Jun 2020		PPQ/Phase 3 (Study P301)
4007220003 (PN: 40072)	5006920001 [redacted]	5006820003 (PN: 50068)	6007520005 (PN: 60075)			30 Jun 2020		PPQ/Phase 3 (Study P301)
4007220003, 4007220004 (PN: 40072)	5006920001 5006920002 [redacted]	5006820003, 5006820004 (PN: 50068)	6007520006 (PN: 60075)			08 Jul 2020		PPQ/Phase 3 (Study P301)
4007220003, 4007220005 (PN: 40072)	5006920001 5006920003 [redacted]	5006820003, 5006820005 (PN: 50068)	6007520007 (PN: 60075)			09 Jul 2020		Phase 3 (Study P301)
4007220003 4007220004	5006920001 5006920002	5006820003 5006820004	6007320001 (Catalent 057G20) (PN: 60073)	Scale A IVT 10 L	0.20	30 Jul 2020	Catalent (Bloomington, IN)	PPQ/Intended for Clinical/EUA Use
4007220003	5006920004	5006820006	6007320002 (Catalent 062G20) (PN: 60073)			06 Aug 2020		PPQ/Intended for Clinical/EUA Use
4007220004 4007220005	5006920005	5006820007	6007320003 (Catalent 001H20) (PN: 60073)			11 Aug 2020		PPQ/Intended for Clinical/EUA Use
4007220005 4007220003	5006920003 5006920007	5006820005 5006820008	6007320004 (Catalent 032H20) (PN: 60073)			13 Sep 2020		Intended for Clinical/EUA Use
4007220002 4007220003 4007220004 4007220005	5006320001 5006320002	5007320002 6007320004	6007320005 (Catalent 011J20) (PN: 60073)	Scale B IVT 10 L (DP = 150,000 vials)	0.20	11 Oct 2020		PPQ/Intended for Clinical/EUA Use

Abbreviations: DP = drug product; LNP = lipid nanoparticle; N/A = not applicable; PN = part number; PVU = personalized vaccine unit, EUA = Emergency Authorization Use; VI = Visual Inspection
DP manufactured in the PVU utilized a sequential integrated process in which CX-024414 mRNA process intermediate is suspended in [redacted] during manufacturing – there is no separate discrete release of the mRNA or an mRNA LNP.

3.2.P.2.3.4 Summary of mRNA-1273 Drug Product Process and Presentation Changes

The first clinical lots of the mRNA-1273 Drug Product (Lots 8520100101, 8520100102, 8520100103, and 850200104) were prepared using the PVU scale process.

After the manufacture of Lot 8520100104, the Scale A ([redacted]) formulation process was implemented. Major changes in mRNA-1273 process and drug product presentation are summarized in Table 4,Table 5 and Table 6.

The comparability demonstration that includes a comparison of parameters and attributes for these manufacturing processes is provided in Section 3.2.P.2.3.7.6. As shown in Table 63 and Table 64 the quality attributes of the mRNA-1273 Drug Product lots are all comparable and meet the specification as defined in Section 3.2.P.5.1.

Table 4: Comparison of Process and Drug Product Presentation Changes mRNA-1273 DP - PVU Process vs Scale A Process (Norwood)

Step	Parameter	PVU Process	Scale A Process Norwood (PN 60075)	Rationale for Change
N/A	Batch Process	Integrated batch from mRNA transcription [redacted] to mRNA 1273 formulation	mRNA-1273 LNP is supplied in [redacted] bags. DP process includes compounding of mRNA-1273 LNP, sterile filtration, filling into vials, and freezing	Meet demand
Dilution	DP Target Concentration	0.5 mg/mL	0.20 mg/mL	To enable 100 mcg per 0.5 mL dose
Fill	Fill Volume	0.6 mL	5.0 mL	Larger fill volume to enable multiple 0.5 mL doses per vial
	Container Closure	Schott 2R Vial with 13 mm PLASCAP	Ompi 10R vial with 20 mm PLASCAP	Larger vial to enable larger fill volume.
Packaging / Labeling	Timing relevant to Fill and Storage	Post-vial inspection Prior to storage.	Flexible **Option 1** Packaged/labeled prior to storage. **Option 2** Packaged and stored. Thawed for labeling. Placed back into storage	To allow for flexibility in labeling and packaging.

Abbreviations: DP = drug product; LNP = lipid nanoparticle; N/A = not applicable

Table 5: Comparison of mRNA-1273 Drug Product Process and Presentation Changes– Scale A Process (Norwood vs Catalent)

Step	Parameter	Scale A Process Norwood (PN 60075)	Scale A Process Catalent (PN 60073)	Rationale for Change
N/A	Facility	Moderna Norwood, MA	Catalent Bloomington, IN	Meet demand
N/A	Batch Scale (Nominal)	[redacted] vials	[redacted] vials	Meet demand
Thawing	N/A	Active Thawing of mRNA-1273 LNP	Passive Thawing of mRNA-1273 LNP	Thaw equipment not available. Passive thawing qualified.
Fill	Fill Volume	5.0 mL	6.3 mL	Fill volume increased to reflect the 10 dose – multiple dose vial presentation.
	Container Closure	Ompi 10R vial with 20 mm PLASCAP	Ompi 10R vial with standard serum stopper and aluminum seal closure	Vial sealing system changed to standard serum stopper and crimp seal to accommodate manufacturing at larger scales. Product contact surface material of stopper is unchanged.
Freezing	Conditioning	None	Initial pre-conditioning freezing at [redacted]	To ensure completion of freezing process prior to -20°C storage
Storage	Temperature	-70˚C (-60˚C to -90˚C)	-20˚C (-15˚C to -25˚C),	Intended long-term storage condition for EUA/commercial presentations

*EUA = Emergency Use Authorization

Table 6: Comparison of mRNA-1273 Drug Product Process and Presentation Changes– Scale A to Scale B Process (Catalent)

Step	Parameter	Scale A Process Catalent (PN 60073)	Scale B Process Catalent (PN 60073)	Rationale for Change
N/A	Manufacturing Scale	45 – 90 L	500 – 1000 L	Meet demand
N/A	Batch Scale (Nominal)	[redacted] vials	[redacted] vials	Meet demand
Thawing	N/A	Passive thawing of mRNA-1273 LNP	Active thawing of mRNA-1273 LNP	Decrease processing time for thaw and increase control of mRNA-1273 LNP thaw.
Visual Inspection	N/A	Manual	Semi-Automated Visual Inspection (SAVI)	Alternatives to manual inspection to decrease processing times and enable large scale manufacturing.

3.2.P.2.3.5 mRNA-1273 Process Characterization

In accordance with ICH Q9, a systematic assessment of the potential risk to mRNA-1273 Drug Product (DP) quality was performed with respect to the manufacturing process. A science-based approach was used to identify Critical Quality Attributes (CQAs), Critical Process Parameters (CPPs), and Critical In-Process Controls (CIPCs) to inform process design studies, and to establish the manufacturing control strategy. The proposed CQAs for mRNA-1273 Drug Product are summarized below in Table 7. The CPPs for the mRNA-1273 Drug Product manufacturing process are provided in Table 2, Section 3.2.P.3.4.1.

This section includes the following to support the development of the mRNA-1273 Drug Product control strategy:

- Description of how the Failure Modes and Effects Analysis (FMEA) of the mRNA-1273 Drug Product manufacturing process was used to identify CPPs and CIPCs and to inform characterization studies (Section 3.2.P.2.3.5.1)
- Characterization data to support the proven acceptable ranges (PARs) for CPPs (Section 3.2.P.2.3.5.2)
- Characterization data to support the PARs for process parameters (Section 3.2.P.2.3.5.3)
- A description of the small-scale models used for characterization studies (Section 3.2.P.2.3.6)

Table 7: mRNA-1273 Drug Product Critical Quality Attributes

Test Category	Attribute	Target Acceptance Criteria
Appearance	Appearance	White to off-white dispersion. May contain visible, white or translucent product-related particulates.
Content	RNA content	[redacted] (Target: 0.20 mg/mL)
Identity	Identity	Sequence matches description
Purity	Purity	[redacted] main peak area
Purity	Product-related impurities	Report % area for each impurity group: Impurity Group 1 (pre-main peak area) Impurity Group 2 (post-main peak area) Impurity Group 3 (mRNA-adduct species)
Encapsulation	% RNA Encapsulation	[redacted]
Potency	In Vitro Translation	
pH	pH	
Osmolality	Osmolality	
Particle Size	Particle size	
PDI	Polydispersity Index	
Lipid Identity	SM-102 Cholesterol DSPC PEG2000-DMG	Matches RT of reference Matches RT of reference Matches RT of reference Matches RT of reference
Lipid Content	SM-102 Cholesterol DSPC PEG2000-DMG	[redacted]
Purity	Lipid Impurities	Individual Impurities: [redacted] area (Report RRTs) Total Impurities: [redacted] area
Particulate Matter	Particulate Matter ≥ 25 µm ≥ 10 µm	[redacted] per container per container
Container Content	Container Content	[redacted]
Bacterial Endotoxin	Bacterial Endotoxins	
Sterility	Sterility	No Growth

Abbreviations: DSPC = 1,2-Distearoyl-sn-glycero-3-phosphocholine; EU = endotoxin unit(s); PDI = polydispersity index; PEG2000-DMG = 1,2-Dimyristoyl-rac-glycero-3-methylpolyoxyethylene; RT = retention time; RRT = relative retention time

3.2.P.2.3.5.1 Failure Modes and Effects Analysis

A FMEA was performed to identify potential failure modes for the manufacturing process and evaluate their potential impact on product quality and/or process performance. Process equipment, process analytical technology, target setpoints, and sensitivity factors for the proposed manufacturing process were considered during the analysis. An analysis was conducted

to evaluate the potential impact of process parameters on mRNA-1273 Drug Product CQAs. A Risk Priority Number (RPN) was determined for each failure mode as the product of the severity, occurrence, and detectability of the potential failure. All high RPN process parameters were evaluated through critical parameter characterization studies to establish PARs or operational controls for the commercial manufacturing process (Section 3.2.P.2.3.5.2). Parameters that were on the threshold of high RPN values were also included in critical parameter characterization studies. Additional parameters that were not identified as high risk by RPN score have been characterized as part of process development activities and are described in Section 3.2.P.2.3.5.3.

3.2.P.2.3.5.2 Characterization Studies for Critical Process Parameters

Proven acceptable ranges for CPPs were characterized using a science- and risk-based approach that leveraged current process understanding and historical knowledge from platform unit operations for similar products. The studies were designed to mitigate potential risks for CPPs identified during the FMEA and establish PARs for the associated process parameters to maintain CQAs.

3.2.P.2.3.5.2.1 Characterization of Dilution Operation

The amount of dilution buffer required to dilute the mRNA-1273 LNP to the mRNA-1273 Drug Product final dilution target concentration is determined from the mRNA content and volume of the clarified mRNA-1273 LNP. The operational controls for the gravimetric dispensing of dilution buffer are adequate to ensure that the mRNA-1273 Drug Product final dilution target concentration is obtained.

The PARs established for this parameter are listed in Table 8.

Table 8: PARs Established for Dilution Operation

Process Variable	PAR	Criticality Designation
Amount of dilution buffer required to dilute the mRNA-1273 LNP to the mRNA-1273 Drug Product final dilution target concentration of 0.20 mg/mL	[redacted]	Critical Process Parameter

Abbreviations: PAR = proven acceptable range

3.2.P.2.3.5.2.2 Characterization of Stoppering and Capping Operation

The container closure integrity was measured on the 10R vials sealed on the Catalent FlexiFill line using a range of capper settings that result in different compression crimp pressures. The integrity of the vials was maintained for all crimp pressures tested at -20°C storage conditions.

The PAR defined for the stoppering and capping operation is shown in Table 9.

Table 9: PARs Established for Stoppering and Capping Operation

Process Variable	PAR	Criticality Designation
Crimp Pressure	[redacted]	Critical Process Parameter

Abbreviations: PAR= proven acceptable range

3.2.P.2.3.5.2.3 Characterization of Cumulative Process Duration

The impact of the cumulative processing duration for time out of refrigeration and at 2 – 8°C was assessed via multiple LNP development hold time studies and batches manufactured at Moderna's Norwood cGMP facility. The use of the cumulative process duration is used in place of intermediate process hold times as the biophysical and chemical changes associated with the bulk LNP [redacted] are assumed to also be representative of the diluted bulk mRNA-1273 Drug Product (target 0.20 mg/mL) as both contain the same formulation. Additionally, a study was performed to show that no individual material used during the mRNA-1273 Drug Product process has an adverse impact on product attributes. mRNA-1273 LNP Samples were held at [redacted] and incubated with individual mRNA-1273 Drug Product manufacturing components/materials of construction. The components were selected based on a manufacturing batch record bill of materials (BOM) similar to those used at Catalent. The full list of evaluated components follows in Table 10. No adverse changes in samples as evaluated by particle size, polydispersity, encapsulation efficiency, sub-visible particles, mRNA content, lipid content, or lipid purity were observed. However, a decrease in mRNA purity was observed for all samples such that no individual material had a specific impact to the mRNA purity.

Table 10: mRNA-1273 Drug Product Manufacturing Components

Manufacturing Material	Vendor	Contact Chemistry
[redacted]		

As the initial hold study demonstrated no expected adverse impact on mRNA-1273 LNP from any specific material, a follow-up study was conducted to determine the impact of extended hold times in the LNP bulk [redacted] bag material. Samples were stored for up to [redacted] at 5°C and 25°C. Results of the second hold time study are provided in Table 11. No substantial changes at either 5°C or 25°C were observed for evaluated biophysical properties, including particle size, polydispersity index, encapsulation efficiency, and sub-visible particle counts. The chemical stability of the mRNA-1273 LNP was impacted by the hold times and was measured in

a separate extended hold-time study, see Table 12. A decrease in mRNA purity was observed at 25°C, with a greatly reduced degradation rate at 5°C. Similarly, an increase in lipid impurities was observed at [redacted] with minimal change at [redacted].

Manufacturing mRNA-1273 Drug Product lots were analyzed and tested to refine the cumulative Time out of refrigeration (TOR) and 2-8°C process duration PARs. The overall mRNA purity loss with relevant processing times from Norwood cGMP development lots are provided in Table 13. Minimal mRNA purity losses are observed for shorter overall processing durations. Extended processing duration hold times were executed for Catalent Scale A mRNA-1273 Drug Product lots, as summarized in Table 14. Minimal changes in mRNA purity, encapsulation, or particle size are observed when the vialed mRNA-1273 Drug Product is held out to longer processing durations. A single Catalent Scale A batch was held out to longer processing hold times to challenge the overall TOR duration to [redacted]. As shown in Table 15, the mRNA-1273 Drug Product is robust to longer processing durations. The PARs defined from these studies and development lots for the cumulative TOR and 2 – 8°C process durations are shown in Table 16. Process duration qualification studies are presented in Section 3.2.P.3.5.

Table 11: Extended mRNA-1273 LNP Liquid Hold Biophysical and Lipid Stability at [redacted]

Storage time (Day)	Particle size (nm)	Polydispersity Index	Encapsulation Efficiency (%)	Sub Visible Particle Counts ([redacted] particles / mL)	Lipid Impurities (%)
[redacted]					

Table 12: Extended mRNA-1273 LNP Liquid Hold mRNA Chemical Stability at 5°C and 25°C

Storage time (Day)	mRNA Purity(%)	
	5°C	25°C
[redacted]		
	Not Tested	[redacted]
	[redacted]	Not Tested
		Not Tested

Table 13: Summary of Cumulative Process Duration and Temperature on mRNA-1273 DP Purity of Development Lots

mRNA-1273 DP Batch	Total Time at 2 – 8°C (h)	Total Time at Room Temperature (h) [a]	mRNA Purity Loss Across DP Processing
6007520001	[redacted]		
6007520002			
6007520003			

Abbreviation: DP = drug product
a) Actual room temperature during the manufacturing of batches listed were controlled to an average of [redacted].

Table 14: Summary of Extended mRNA-1273 DP Processing Holds on Product Quality

mRNA-1273 Batch	057G20			062G20			001H20		
Sample Description	mRNA-1273 LNP CoA [a]	Drug Product CoA	Extended Hold Sample	mRNA-1273 LNP CoA	Drug Product CoA	Extended Hold Sample	mRNA-1273 LNP CoA	Drug Product CoA	Extended Hold Sample
Cumulative Time at CRT	N/A	[redacted]		N/A	[redacted]		N/A	[redacted]	
Cumulative Time at 5°C	N/A			N/A			N/A		
mRNA Purity (%)	[redacted]								
% Encapsulation									
Particle Size (nm)									
PDI									

a) Two mRNA-1273 LNP lots were polled together for mRNA-1273 DP lot 057G20. The tabulated values are from COA 5006820003 and 5006820004

Table 15: Summary of Extended TOR mRNA-1273 Drug Product Processing Holds on Product Quality

mRNA-1273 Batch	032H20		
Sample Description	mRNA-1273 LNP CoA	Drug Product CoA	Extended Hold Sample
Cumulative Time at CRT	N/A	[redacted]	
Cumulative Time at 5°C	N/A		
mRNA Purity (%)	[redacted]		
% Encapsulation			
Particle Size (nm)			

Table 16: Cumulative Processing Durations

Process Variable	PAR	Criticality Designation
Cumulative time out of refrigeration (TOR, [redacted])	[redacted]	Critical Process Parameter
Cumulative processing duration at 2 – 8°C		Critical Process Parameter

Abbreviations: PAR = proven acceptable range; TOR = time out of refrigeration

3.2.P.2.3.5.2.4 Characterization of Vial Freezing and Storage – Conditioning Temperature

A vial pre-conditioning step at a target temperature of [redacted] is used to ensure the mRNA-1273 Drug Product vials are completely frozen prior to stationing at the long-term storage temperature of -20°C. If the conditioning temperature is lower than the T_g of the stopper (approximately [redacted]), the container closure integrity may be at risk. The PARs defined for the vial conditioning temperature are shown in Table 17.

Table 17: Vial Conditioning Temperature

Process Variable	PAR	Criticality Designation
Vial Conditioning Temperature	[redacted]	Critical Process Parameter

3.2.P.2.3.5.3 Characterization Studies for Process Parameters Classified as Not Critical

Process parameters classified as not critical (based on potential for CQA impact) were characterized to verify lack of impact on process performance. These parameters were either identified as low risk during the FMEA or were determined to not be critical based on the results of the characterization studies described below which did not show statistically significant or practically significant effects on any mRNA-1273 LNP CQAs.

3.2.P.2.3.5.3.1 Characterization of Thawing Operation – Thaw Rate

The thawing operation was characterized to evaluate the impact of thawing rate on mRNA-1273 Drug Product CQAs. The bulk mRNA-1273 LNP was put through five freeze-thaw (F/T) cycles using a series of temperature programs designed to mimic a range of process conditions. The experiments used a scale down model (SDM) that was developed to bracket both passive (slow freeze/slow thaw) and active processess (representative freeze/thaw and fast freeze/fast thaw). Details of the SDM are provided in Section 3.2.P.2.3.6. Each experiment was characterized for appearance, hydrodynamic diameter and polydispersity index (PDI), mRNA encapsulation efficiency (% EE), and sub-visible particulates.

The PARs established by the study are listed in Table 18. The data demonstrating the impact of thawing PARs on CQAs are presented in Table 19. No impact was observed for appearance, particle size, polydispersity, and % EE after 5 F/T cycles. An increase in sub-visible particulates was observed after the fifth F/T cycle, but still within normal operating ranges. Results after the [redacted] F/T cycle were minimally changed from the [redacted] F/T cycle. These results show that the product is not sensitive to variations in thawing temperature and duration and is stable for up to [redacted] F/T cycles.

Table 18: PARs Established from Thawing Operation Study

Process Variable	Target	PAR
pFTU shelf temperature program	[redacted]	N/A
Thaw Duration	[redacted]	[redacted]
Post thaw intermediate pFTU shelf temperature set point	[redacted]	[redacted]
Post thaw intermediate storage temperature in CTU	[redacted]	[redacted]

Abbreviations: PAR = proven acceptable range; pFU = plate freeze=thaw unit; CRT = controlled room temperature ([redacted])

Table 19: Impact of Multiple Freeze-Thaw Cycles on mRNA-1273 Drug Product Attributes

Number of F/T Cycles	Particle Size by DLS (nm) (Specification: [redacted])			Encapsulation Efficiency by RiboGreen (%) (Specification: [redacted])			Subvisible Particle Counts ([redacted])		
	Arm 1: SF/ST	Arm 2: FF/FT	Arm 3: [redacted] SDM	Arm 1: SF/ST	Arm 2: FF/FT	Arm 3: [redacted] SDM	Arm 1: SF/ST	Arm 2: FF/FT	Arm 3: [redacted] SDM
[redacted]									

Abbreviations: DLS = dynamic light scattering; SF/ST = slow freeze/slow thaw; FF/FT = fast freeze/fast thaw; SDM = scale down model; F/T= freeze/thaw; NT = not tested

3.2.P.2.3.5.4 Characterization of Thawing Operation -Shelf Temperature Program

The thawing recipe for the [redacted] single-use bag assembly was developed during installation and operational qualification (IOQ) of the Single use Support (SUS) large-scale plate freeze-thaw unit (pFTU). [redacted] single-use bag assemblies were filled with [redacted] and placed into Robust Shipping and Storage (RoSS) shells. The thawing shelf program was selected based on vendor recommendations. The thawing profiles of the average product temperature for each IOQ run are shown in Figure 2. All three IOQ thawing profiles reach a bulk solution bag temperature above [redacted]. The data supports the PAR for thawing duration in Table 18.

Figure 2: Thawing Profiles from IOQ Runs Using [redacted] in the pFTU Using a Program Shelf Temperature Recipe

[redacted]

3.2.P.2.3.5.5 Characterization of Clarification and Sterile Filtration Operation – Filter Loading

Both clarification and sterile filtration use [redacted] filters [redacted] filtration assemblies. Clarification is performed on the thawed mRNA-1273 LNP at a nominal concentration of [redacted]. Sterile filtration is performed on the 0.20 mg/mL diluted mRNA-1273 bulk drug product. Filters for manufacturing are sized based on the acceptable mRNA filter loading per unit filter surface area (g mRNA/m^2) and associated batch size. The filter pressure is monitored throughout the process to ensure it is below the [redacted] validated during bacterial challenge testing to ensure the production of a sterile effluent. The filtration flux (i.e. flowrate) may be reduced during the process as needed to maintain the pressure [redacted]. The filters are received pre-sterilized. The sterile filters are assembled, tested for integrity with WFI, and blown down with compressed air prior to use. Post-use integrity testing is performed offline after rinsing the filters with a [redacted].

The PARs established for the clarification filtration and sterile filtration operation are summarized in Table 20 and Table 21, respectively. Data supporting the process parameter of filter loading for the sterile filtration operation from development product batches are summarized in Table 22. A lab-scale experiment evaluated the filtration performance of GMP lot 5007320002 using [redacted]. The study found that during clarification and sterile filtration, the pressure reached [redacted], respectively (see Figure 3). The data supports the PARs for sterile filtration filter loading in Table 21.

Table 20: PARs Established for Clarification Filtration Operation

Process Variable	PAR	Criticality Designation
Filtration flux	[redacted]	Process Parameter
Maximum filtration pressure		In-Process Control
Post-use filter integrity test		In-Process Control

Abbreviation: PAR = proven acceptable range, PUPSIT = pre use post sterilization integrity testing; WFI = water for injection

Table 21: PARs Established for Sterile Filtration Operation

Process Variable	PAR	Criticality Designation
Filtration Size / Membrane Loading [a]	[redacted]	Process Parameter
Pre-use filter integrity test (vendor COA, gamma irradiated)		In-Process Control
Post-PUPSIT blow-down pressure		Process Parameter
Post-PUPSIT blow-down duration		Process Parameter
Filter Flush Volume [b]		Process Parameter
Filtration flux		Process Parameter
Maximum filtration pressure		Critical In-process Control
Post-use filter integrity test		Critical In-process Control

Abbreviation: PAR = proven acceptable range, PUPSIT = pre use post sterilization integrity testing; WFI = water for injection

a) Filter size will be selected based on batch size to a target loading of [redacted]. The filter loading may be exceeded during processing as long as the measured pressure is controlled [redacted].

b) The total filter flush volume is [redacted]

Table 22: Maximum Pressure for Sterile Filtration for Production Batches

Batch Number	mRNA Concentration (mg/mL)	Filter Loading (g mRNA/m^2)	Filtration Flux (L/min/m^2)	Filtration Flow Rate (mL/min)	Maximum Pressure (psi)
F002720001	[redacted]				
F002720002					
F002720003					

Figure 3: Filtration Pressure Profiles for an Aliquot of Lot 5007320002 that was Frozen, Thawed, and Processed Using [redacted] Filter Capsules

[redacted]

3.2.P.2.3.5.6 Characterization of Pooling and Dilution Operation – Mixing Speed and Duration

The PARs established for the pooling and dilution mixing operations are summarized in Table 23. The mixing parameters for each batch are dependent on the mixing vessel. Mixing studies were performed during manufacturing of Catalent Scale A PPQ lots during pooling and dilution as described in Table 24. The mixing rates were varied as part of mixing studies. The pooling operation mixing parameters represent a greater risk to product quality impact compared to the dilution operation mixing parameters. Table 25 shows that the listed product quality attributes are not changed from the pre-freeze mRNA-1273 LNP and post-pooling mixing bulk mRNA-1273 LNP. The dilution operation mixing parameter represent a greater risk to product uniformity throughout the bag. Samples were taken from the top and bottom of the mixing bag for each PPQ lot at the [redacted] mixing durations. The diluted mRNA-1273 bulk Drug Product pH and osmolality for each sample pool are summarized in Table 26. Each sample meets the expected acceptance criteria for pH, osmolality, and mRNA concentration with no changes from [redacted] mixing durations indicating a well-mixed uniform product. Mixing studies should be performed as new mixing vessels are used to support mRNA-1273 Drug Product manufacturing.

Table 23: PARs Established for Pooling and Dilution Mixing Operations

Process Variable	PAR	Criticality Designation
Mixing Volume	[redacted]	Process Parameter
Mixer Speed		Process Parameter
Pooling Mixing Duration		Process Parameter
Dilution Mixing Duration		Process Parameter

Table 24: Mixing Parameters in Catalent Scale A Batches

PPQ Batch	Unit Operation	mRNA-1273 Concentration (mg/mL)	Mixing Vessel	Mixing Speed (rpm)	Mixing Time (min)	Liquid Volume (L)
6007320001	Pooling	[redacted]				
057G20	Dilution					
6007320002	Pooling					
062G20	Dilution					
6007320003	Pooling					
001H20	Dilution					

Table 25: Pooling Mixing Catalent Scale A Product Quality Results

Analytical Assay	6007320001 (057G20)			6007320002 (062G20)		6007320003 (001H20)	
	Pre-Freezing [a]	Pre-Freezing [b]	Post-Pooling	Pre-Freezing	Post-Pooling	Pre-Freezing	Post-Pooling
mRNA Content (mg/mL)	[redacted]						
mRNA Purity (% Main Peak)							
Mean Particle Size (nm)							
PDI							
RNA Encapsulation (%)							
Lipid Impurities (%)							
Subvisible Particles (particles/mg)							

Abbreviations: PDI = polydispersity index; NT = not tested
a, b) The first PPQ Batch was composed of two different LNP lots with approximately [redacted] coming from (a) and [redacted] coming from (b).

Table 26: Dilution Mixing Operation Uniformity Results

Lot	Mixing Time	[redacted]					
	Mixer Position	Top	Bottom	Top	Bottom	Top	Bottom
	Mixer Speed	[redacted]					
6007320001 057G20	pH						
	Osmolality (mOsm/kg)						
	mRNA Concentration (mg/mL)						
6007320002 062G20	pH						
	Osmolality (mOsm/kg)						
	mRNA Concentration (mg/mL)						
6007320003 001H20	pH						
	Osmolality (mOsm/kg)						
	mRNA Concentration (mg/mL)						

3.2.P.2.3.5.7 Characterization of Vial Freezing and Storage – Conditioning Duration

A [redacted] conditioning freeze step is used in order to ensure the vials are in the frozen state prior to long-term storage at -20°C. Development studies at Moderna freezing [redacted] vials of 0.20 mg/mL mRNA-1273 at [redacted] in 10R vials using a [redacted] freezer demonstrated that all vials reached [redacted]. This temperature is extremely conservative and freezing studies should be performed at the manufacturing site to confirm the recommended freezing duration of [redacted].

3.2.P.2.3.6 Scale Down Model(s)

3.2.P.2.3.6.1 Bulk Thawing Scale Down Model

A scale down model (SDM) was developed to facilitate studying the impact of both active thawing in a controlled plate freeze-thaw unit (pFTU) and passive thawing on the quality attributes of LNPs. In an active thawing process conducted on the pFTU, the temperature of the heat transfer plates is fixed but the overall thaw duration will vary based on the volume of solution in the bag. For a passive process, the lack of an active transfer of heat to the bag by conduction results in longer thaw durations for comparable volumes relative to the active process. The SDM was developed to simulate three conditions that the bulk mRNA-1273 LNPs could experience: a fast-freeze/fast-thaw (FF/FT) process corresponding to a low fill volume in an active pFTU, a representative process ([redacted]) corresponding to a maximum fill volume in an active pFTU, and a slow-freeze/slow-thaw (SF/ST) process corresponding to a maximum fill volume in a passive process. Each condition was designed to mimic the processing time the at scale bulk product spends in a transitional ice-water phase between 0°C and the glass transition temperature (Tg', approximately [redacted]) during both freezing and thawing (Figure 4).

Figure 4: Ice-Water Transitional Time for Various Freeze-Thaw Processes

[redacted]

Abbreviations: SF/ST = slow freeze/slow thaw; FF/FT = fast freeze/fast thaw; SDM = scale down model; F/T = freeze/thaw

3.2.P.2.3.6.2 Filtration Scale Down Model

A scale-down model (SDM) for the mRNA-1273 Drug Product clarification and sterile filtration operations was developed to ensure that the mRNA-1273 Drug Product CQAs were maintained within specification and the process parameters correlated to the relevant commercial scale parameters. The design elements of the SDM and the corresponding commercial scale parameters are provided in Table 27.

Table 27: Comparison of Scale Down Model and Commercial Scale Parameters for Filtration Operations

Unit Operation	Parameter	[redacted] Vial Scale Target	Scale Down Model Target
Clarifying Filtration	Filtration loading (filter area)	[redacted]	
	Filtration flux (pump flow rate)		
	Filtration pressure		
Sterile Filtration	Filtration loading (filter area)		
	Filtration flux (pump flow rate)		
	Filtration pressure		

3.2.P.2.3.6.3 Shear Stress Scale Down Model

A scale-down model was developed to evaluate the impact of both shear rate (due to flow rates or mixing speeds) or total shear (due to tubing length or mixer residence times) on the mRNA-1273 Drug Product CQAs. Shear rates and total shear for various manufacturing processes are described in the literature (Ogunyankin et al, 2019) and are summarized in Table 28.

Table 28: Typical Shear Conditions Encountered in Manufacturing Processes

Unit Operation	Shear Rate (s^{-1})	Total Shear (dimensionless)
Mixing	[redacted]	
Tubing		
Filtration		
Pumping		
Filling (needle)		

The SDM utilizes a capillary shear device, in which a syringe pump is used to pump mRNA-1273 Drug Product through capillary tubing of varying lengths and diameters at varying flowrates. The model was developed to cover the full range of potential shear conditions (Table 28) using a full factorial multivariate study. No impact to particle size, mRNA encapsulation, sub-visible particulates, or purity was observed over any of the conditions evaluated. These results demonstrate that typical assumed shear conditions encountered during standard unit operations have no impact on mRNA-1273 Drug Product CQAs.

3.2.P.2.3.6.4 Interfacial Stress Scale Down Model

Interfacial stress is a physical stress arising at a boundary between two phases, such as fluid/solid or liquid/gas. The mRNA-1273 Drug Product hydrophobic moieties within the nanoparticles may adsorb to liquid-air interfaces and cause the loss of structural integrity. The mRNA-1273 manufacturing process may expose the mRNA-1273 Drug Product to these interfaces primarily during mixing and filling. It is not convenient, however, to directly determine the impact of interfacial stress in full-scale unit operations.

The interfacial stress scale-down model utilizes a controlled sparging device in which a peristaltic pump delivers a steady flow of gas that bubbles through a thin capillary into the vial of the liquid to be tested. The bubbling rate (controlled by pump flow rate) and bubble size (capillary dimensions) can be measured via high-speed video during operation. Since the pump delivers a constant rate of bubbles, the total interfacial area may be estimated as a function of sparging time. General assumption to estimate representative interfacial stress conditions encountered in mixing and filling processes, as shown in Table 29. These ratios were tested and exceeded in the scale-down device.

Table 29: Estimated Interfacial Stress Conditions Encountered in mRNA-1273 Drug Product Manufacturing Process

Unit Operation	Interface Generated, S (cm^2)	Bulk Liquid Volume, V (mL)	Interface to Volume Ratio, S/V (cm^{-1})	Interface to Lipid Ratio, S/M (cm^2/mg)
Mixing	[redacted]			
Filling				

Abbreviations: DP = drug product; M = lipid mass; S = surface area; V = volume

Low levels of interfacial stresses assumed to be representative of typical manufacturing processes were not found to impact the biophysical or chemical properties of the mRNA-1273 Drug Product. However, at greatly elevated levels of interfacial stress [redacted], the mRNA-1273 Drug Product experienced changes in particle size, encapsulation efficiency, and sub-visible particle counts. As such, the impact of interfacial stress from typical manufacturing processes is assumed to be minimal; however, care should be taken to avoid excessive air entrapment during mixing or filling to mitigate potential product quality risk.

3.2.P.2.3.7 Analytical Assessment

The goal of this analytical assessment was to demonstrate that the quality attributes of the product are highly similar across processes and that the existing knowledge is sufficiently predictive to ensure that any differences in quality attributes have no adverse impact upon safety or efficacy of the drug product according to *ICH Guideline Q5E: Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process*. Included in the assessment is 1) release, 2) stability, and 3) extended characterization. The first manufacturing train at the Sponsor's Norwood facility, for PPQ batch consistency assessment will be used as the baseline to establish commercial specification and additional product characteristics using orthogonal characterization assays.

PPQ activities with the aim to enable a commercial manufacturing process for mRNA-1273 have been initiated, while concurrently conducting the Phase 2 and Phase 3 human clinical trials. PPQ activities were carried out at GMP manufacturing facilities at ModernaTX, Inc. in Norwood, MA [redacted], the manufacturing site for clinical trial materials, and Catalent Biologics, LLC in Bloomington, IN [redacted]. As part