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3.2.S.2.5 PROCESS VALIDATION AND/OR EVALUATION

A Process Validation Master Plan was developed to ensure that the commercial manufacturing process for CX-024414 mRNA will reliably and consistently produce product of appropriate quality.

Product development of CX-024414 mRNA was accelerated in response to the COVID-19 pandemic. To accelerate the collection of scientific data that demonstrate process consistency, the process performance qualification (PPQ) was executed concurrently with process development activities. The process parameters and proven acceptable ranges (PARs) evaluated during PPQ were determined prior to the completion of the process development activities; the process development studies are discussed in Section 3.2.S.2.6 {CX-024414}, for 20 L in vitro transcription (IVT) scale. The final determination of critical process parameters (CPPs) and PARs will be based on the results of PPQ and the process development data and summarized in Section 3.2.S.2.6 {CX-024414}.

Three PPQ lots (20 L IVT scale) were completed at Lonza Biologics, Inc. in Portsmouth, NH to demonstrate process consistency for CX-024414 mRNA manufacturing process (Table 1).

PPQ for the 60 L IVT scale CX-024414 mRNA manufacturing process at Lonza Biologics, Inc. (Portsmouth, NH, USA) is currently on-going. The process parameters and PARs were determined prior to the initiation of PPQ activities. The process performance protocol for the 60 L IVT scale, USPO-27961, has been provided.

A minimum of 3 PPQ lots is required if a new process is established, a new scale is established, or an existing, qualified process is transferred to a new site. For a given qualified process, at a given site, additional process trains will be qualified with one additional PPQ lot and compared to previous PPQ lots for that process.

Table 1: CX-024414 mRNA Process Performance Qualification Lots

Manufacturing Location	Scale	Moderna Lot Number (Lonza Lot Number)	Date of Manufacture
Lonza, Biologics, Inc. Portsmouth, NH	20 L	4007520002 (922693)	12-Aug-20
Lonza, Biologics, Inc. Portsmouth, NH	20 L	4007520003 (928769)	25-Aug-20
Lonza, Biologics, Inc. Portsmouth, NH	20 L	4007520004 (930143)	31-Aug-20

For the 20 L IVT PPQ scale at Lonza (Portsmouth, NH), all process parameters and in-process controls defined in Section 3.2.S.2.2 {CX-024414} were evaluated during the PPQ and confirmed to be within target ranges. Noted exceptions did not have impact on product quality or the execution of the PPQ protocol. Each unit operation of the CX-024414 mRNA manufacturing process and the corresponding PPQ results are summarized in the following subsections. Continuous data that met the expected range are presented as “Conforms”, with

full details available in the summary report attached in this section, USPO-27963. The summary protocol, USPO-27364 is provide as an attachment. Detailed descriptions of the manufacturing process, including the process flow diagram, are provided in Section 3.2.S.2.2 {CX-024414}. The manufacturing controls are defined in Section 3.2.S.2.4 {CX-024414}. All critical quality attributes and specifications provided in Section 3.2.S.4.1 {CX-024414}.

Excursions outside of the process parameter and in-process control ranges were investigated per deviation procedure and evaluated to confirm no product impact as a result of the excursions. in Section 4.2 in USPO-27963.

The process performance qualifications, performed under protocol, met the following acceptance criteria:

- A minimum of 3 consecutive batches meeting specifications for all critical quality attributes and critical process parameters meeting acceptance criteria.
- Assessment of any non-critical process parameters and in-process controls that fail to meet the pre-established parameter ranges, with confirmation of no impact to the performance of the unit operation or the validity of the qualification.
- Description of any validation protocol exceptions, with confirmation of no impact to the validity of the qualification.

Full details of the PPQ are available in summary reports USPO-27963 (20 L IVT scale). A Summary of PPQ Step Yield Data is provided as a separate attachment.

3.2.S.2.5.1 Performance Qualification Results for 20 L IVT Scale by Unit Operation

3.2.S.2.5.1.1 In Vitro Transcription

All PPQ lots for this unit operation met the pre-established ranges, as shown in Table 2, demonstrating a controlled process ready for commercial production.

Table 2: In Vitro Transcription Process Performance Qualification Results

Process Variable	Target/Range	Results		
	20 L IVT	4007520002 (Lonza Lot 922693)	4007520003 (Lonza Lot 928769)	4007520004 (Lonza Lot 930143)
CCI	CCI			
		Conforms	Conforms	Conforms
CCI	CCI			
		Conforms	Conforms	Conforms
CCI	CCI			

Abbreviations: ATP = adenosine triphosphate; CTP = cytidine triphosphate; EDTA = ethylenediaminetetraacetic acid; GTP = guanosine triphosphate; IVT = in vitro transcription; N1-Me-ΨTP = N1-methylpseudouridine triphosphate; PPiase = pyrophosphatase, inorganic; TFF = tangential flow filtration

a) Parameters with conforms are confirmed via trend review during PPQ data analysis.

3.2.S.2.5.1.2 In Vitro Transcription Tangential Flow Filtration

All PPQ lots for this unit operation met the pre-established ranges, as shown in Table 3, demonstrating a controlled process ready for commercial production.

Table 3: In Vitro Transcription Tangential Flow Filtration Process Performance Qualification Results

Process Variable	Target/Range	Results		
	20 L IVT	4007520002 (Lonza Lot 922693)	4007520003 (Lonza Lot 928769)	4007520004 (Lonza Lot 930143)
[redacted]		Conforms	Conforms	Conforms
		[redacted]		
		Conforms	Conforms	Conforms
		Conforms	Conforms	(b)
		[redacted]		
		Conforms	Conforms	Conforms
		[redacted]		

Abbreviations: FIT = filter integrity test; IVT = in vitro transcription; TFF = tangential flow filtration; TMP = transmembrane pressure

a) Parameters with conforms are confirmed via trend review during PPQ data analysis.

b) Deviation occurred (refer to TW703110 in Section 4.2 in USPO-27963).

3.2.S.2.5.1.3 First Oligo dT Chromatography

All PPQ lots for this unit operation met the pre-established ranges, as shown in Table 4, demonstrating a controlled process ready for commercial production. A resin lifecycle of up to CCI has been established as shown in Section 3.2.S.2.4.4 {CX-024414}.

Table 4: First Oligo dT Chromatography Process Performance Qualification Results

Process Variable	Target/Range	Results		
	20 L IVT	4007520002 (Lonza Lot 922693)	4007520003 (Lonza Lot 928769)	4007520004 (Lonza Lot 930143)
CCI		CCI		
		Conforms	Conforms	Conforms
		Conforms	Conforms	Conforms
		CCI		
		N/A	N/A	N/A
		Conforms	Conforms	Conforms
		Conforms	**(b)**	Conforms
		CCI		

Abbreviations: AU = absorbance unit(s); CV = column volume(s); dT = deoxythymidine
a) Parameters with conforms are confirmed via trend review during PPQ data analysis.
b) Deviation occurred (refer to DR-01 in Section 4.1 of USPO-27963).

3.2.S.2.5.1.4 dT Tangential Flow Filtration

All PPQ lots for this unit operation met the pre-established ranges, as shown in Table 5, demonstrating a controlled process ready for commercial production. Membrane reuse throughout a batch has been established as described in Section 3.2.S.2.4.4 {CX-024414}.

Table 5: dT Tangential Flow Filtration Process Performance Qualification Results

Process Variable	Target/Range	Results		
	20 L IVT	4007520002 (Lonza Lot 922693)	4007520003 (Lonza Lot 928769)	4007520004 (Lonza Lot 930143)
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	Conforms	Conforms	Conforms
[redacted]	[redacted]	Conforms	Conforms	Conforms
[redacted]	[redacted]	Conforms	(c)	(c)
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	N/A	N/A	N/A
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Abbreviations: dT = deoxythymidine; NWP = normalized water permeability; TFF = tangential flow filtration; TMP = transmembrane pressure

a) Parameters with conforms are confirmed via trend review during PPQ data analysis.

b) Data reported as an absolute value. Data meets target/range of [redacted] of initial NWP

c) Deviation occurred (refer to TW703110 in Section 4.2 of USPO-27963).

3.2.S.2.5.1.5 Capping

All PPQ lots for this unit operation met the pre-established ranges, as shown in Table 6, demonstrating a controlled process ready for commercial production.

Table 6: Capping Process Performance Qualification Results

Process Variable	Target/Range	Results		
	20 L IVT	4007520002 (Lonza Lot 922693)	4007520003 (Lonza Lot 928769)	4007520004 (Lonza Lot 930143)
CCI [redacted]		CCI [redacted]		
		Conforms	Conforms	Conforms
		CCI [redacted]		

Abbreviations: dT = deoxythymidine; GTP = guanosine triphosphate; SAM = s-adenosylmethionine; TFF = tangential flow filtration
a) Parameters with conforms are confirmed via trend review during PPQ data analysis.
b) Deviation occurred (refer to TW697473 in Section 4.2 of USPO-27963).

3.2.S.2.5.1.6 Cap Tangential Flow Filtration

All PPQ lots for this unit operation met the pre-established ranges, as shown in Table 7, demonstrating a controlled process ready for commercial production. Membrane reuse throughout a batch has been established as shown in Section 3.2.S.2.4.4 {CX-024414}.

Table 7: Cap Tangential Flow Filtration Process Performance Qualification Results

Process Variable	Target/Range	Results		
	20 L IVT	4007520002 (Lonza Lot 922693)	4007520003 (Lonza Lot 928769)	4007520004 (Lonza Lot 930143)
CCI		CCI		
		Conforms	Conforms	Conforms
		Conforms	Conforms	Conforms
		Conforms	Conforms	**(c)**
		CCI		
		Conforms	Conforms	Conforms
		CCI		

Abbreviations: NWP = normalized water permeability; TFF = tangential flow filtration; TMP = transmembrane pressure
a) Parameters with conforms are confirmed via trend review during PPQ data analysis.
b) Data reported as an absolute value. Data meets target/range of CCI of initial NWP
c) Deviation occurred (refer to TW703110 in Section 4.2 in USPO-27963).

3.2.S.2.5.1.7 Second Oligo dT Chromatography

All PPQ lots for this unit operation met the pre-established ranges as shown in Table 8, demonstrating a controlled process ready for commercial production. A resin lifecycle of up to CCI has been established as shown in Section 3.2.S.2.4.4 {CX-024414}.

Table 8: Second Oligo dT2 Chromatography Process Parameters

Process Variable	Target/Range	Results		
	20 L IVT	4007520002 (Lonza Lot 922693)	4007520003 (Lonza Lot 928769)	4007520004 (Lonza Lot 930143)
CCI		Conforms	Conforms	**(b)**
		Conforms	Conforms	Conforms
		CCI		
		Conforms	Conforms	Conforms
		Conforms	Conforms	Conforms
		Conforms	Conforms	Conforms
		CCI		

Abbreviations: AU = absorbance unit(s); CV = column volume(s); dT = deoxythymidine
a) Parameters with conforms are confirmed via trend review during PPQ data analysis.
b) Deviation occurred (refer to TW703780 in Section 4.2 in USPO-27963).

3.2.S.2.5.1.8 Final Tangential Flow Filtration

All PPQ lots for this unit operation met the pre-established ranges, as shown in Table 9, demonstrating a controlled process ready for commercial production. Membrane reuse throughout a batch has been established as shown in Section 3.2.S.2.4.4 {CX-024414}.

Table 9: Final Tangential Flow Filtration Process Parameters

Process Variable	Target/Range	Results		
	20 L IVT	4007520002 (Lonza Lot 922693)	4007520003 (Lonza Lot 928769)	4007520004 (Lonza Lot 930143)
CCI		CCI		
		Conforms	Conforms	Conforms
		Conforms	Conforms	Conforms
		Conforms	Conforms	**(c)**
		CCI		
		Conforms	N/A	N/A
		CCI		

Abbreviations: NWP = normalized water permeability; TFF = tangential flow filtration; TMP = transmembrane pressure
a) Parameters with conforms are confirmed via trend review during PPQ data analysis.
b) Data reported as an absolute value. Data meets target/range of CCI of initial NWP
c) Deviation occurred refer (refer to TW703110 in Section 4.2 in USPO-27963).

3.2.S.2.5.1.9 Clarification, Post-Clarification Hold, and Storage

All PPQ lots for this unit operation met the pre-established ranges as shown in Table 10, demonstrating a controlled process ready for commercial production.

Table 10: Clarification, Post-Clarification Hold, and Storage Process Parameters

Process Variable	Target/Range	Results		
	20 L IVT	4007520002 (Lonza Lot 922693)	4007520003 (Lonza Lot 928769)	4007520004 (Lonza Lot 930143)
CCI	Conforms	Conforms	Conforms	Conforms
	Conforms	Conforms	Conforms	Conforms
	CCI			
	Final Storage	Final Storage	Final Storage	Final Storage

Abbreviations: TFF = tangential flow filtration
a) Parameters with conforms are confirmed via trend review during PPQ data analysis.
b) Per filter vendor validation guide, Forward Flow method is appropriate for correlation to microbial retention.

3.2.S.2.5.1.10 Process Performance Qualification Deviations for 20L IVT Scale

Protocol discrepancies are summarized in Table 11. Deviations that occurred during the execution of the PPQ are summarized in Section 4.2 in USPO-27963. No noted discrepancy impacts the PPQ acceptance criteria.

Table 11: Protocol Discrepancies

Discrepancy #	Description	Impact Assessment
20L IVT PPQ		
DR-01	Additional parameters were reported in the summary report.	There was no product impact; this event was documentation related only.
DR-02	Expected transition/stabilization periods were not specifically called out in the 5156 Process Description, USPO-27260. Stabilization periods were consistently observed throughout the PPQ series. The terminology "conforms" was reported if the acceptable range was met, in place of reporting minimum, maximum and mean values.	There was no product impact. All parameters in scope of the discrepancy were found to be within the specified acceptable ranges for the duration of the operation, following the initial stabilization period, unless otherwise noted in the tables with a deviation reference. There was no product impact as a result of using different reporting terminology; it was documentation related only.
DR-03	Residual Protein by CCI was unable to be reported for any of the Tangential Flow Filtration (Final TFF) lots.	There was no product impact as a result of this discrepancy. Residual Protein by CCI was tested upon completion of the process step which occurs prior to Final TFF (CCI). Results were within specification CCI Acceptable Range) for all PPQ lots.

3.2.S.2.5.1.11 Process Performance Qualification In-Process and Release Testing Results for 20 L IVT Scale

3.2.S.2.5.1.11.1 In-process Testing

All PPQ lots met the predefined in-process control acceptable ranges, as shown in Table 12.

Table 12: In-Process Controls Results

Attribute	Method	Sample Point	Acceptable Range	Results		
			20 L IVT	4007520002 (Lonza Lot 922693)	4007520003 (Lonza Lot 928769)	4007520004 (Lonza Lot 930143)
CCI						

Abbreviations: dT = deoxythymidine; IVT = in vitro transcription; TFF = tangential flow filtration

3.2.S.2.5.1.11.2 Release Testing

All release testing results, encompassing all Critical Quality attributes, met the predefined acceptance criteria for each PPQ lot and are summarized in Section 3.2.S.4.4 {CX-024414}. All release testing was performed with qualified analytical procedures.

3.2.S.2.5.1.12 Process Performance Qualification Conclusion for 20L IVT Scale

The PPQ met all of acceptance criteria provided in the PPQ protocol as provided in Table 13. Three consecutive batches were completed which met all specifications. All in-process controls met the pre-established ranges. All process parameters met the pre-established parameter ranges, with exceptions listed and discussed in Table 11 for protocol discrepancies and for deviations as discussed in Section 4.2 in USPO-27963.

Table 13: PPQ Acceptance Criteria and Conclusion

Category	Acceptance Criteria	Conclusion
Consistency	A minimum of three consecutive batches meeting specifications for all critical quality attributes (CQAs) will be completed to demonstrate consistency and reproducibility. Non-consecutive batches, separated by failed batches whose failure can be attributed to a non-process related cause, are acceptable.	Process performance qualification was performed for three consecutive batches at each scale identified in Table 1. All CQAs were met and no non-consecutive batches were executed.
Robustness	Process parameters and in-process controls (IPC) that fail to meet the pre-established parameter ranges must be assessed and determined to not impact the consistent performance of the unit operation and the validity of the study.	All process parameters and in-process controls defined in the respective Technology Transfer documents for each scale identified in Table 1 were evaluated. Potential batch impacting deviations were noted as described in the respective PPQ Summary Reports. The noted deviations were deemed to not impact product quality nor the performance consistency of the unit operation.
Robustness	Unit operations may be validated independently, if justified. Any failure of a CQA specification to meet batch release requirements must be shown to be unrelated to the unit operation being validated.	No CQA failures occurred during execution of this process performance qualification.
Compliance	Any validation protocol exceptions must be described in the final report and determined not to impact the validity of the study.	Three protocol discrepancies were noted as identified in Table 11. No discrepancies impacted the validity of the study.