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Method Validation Report

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Compendia Validation Report

CX-024414 mRNA, mRNA-1273 LNP, mRNA-1273 DP, [redacted]

1. Introduction

This report presents a summary for the verification of compendial test methods used for CX-024414 mRNA, mRNA-1273 Lipid Nanoparticle (LNP), mRNA-1273 Drug Product (DP), [redacted]

The verification of each compendial method is in accordance with Current USP <1226> Verification of Compendial Procedures, such that this report documents evidence of suitability under actual conditions of use. Each compendial method has been assessed as appropriate to the complexity of the procedure.

The compendial test methods have been assessed for suitability using representative sample types and deemed appropriate for testing CX-024414 mRNA, mRNA-1273 Lipid Nanoparticle (LNP), mRNA-1273 Drug Product (DP), [redacted] sample types as summarized in the table below.

Compendial Test Method	SOP #	CX-024414 mRNA	mRNA-1273 LNP	mRNA-1273 DP	[redacted]
Appearance	SOP-0278	X	X	X	X
pH	SOP-0288	X	X	X	X
Osmolality	SOP-0279		X	X	X
Container Content	SOP-0950			X	
Particulate Matter	SOP-0509			X	
Residual Solvents	MRA-C0000-GTM0018				X

2. Responsibilities

Department/ Functional Area	Responsibility
Quality Control	• Authors, reviews and approves validation protocols and reports. • Executes, reviews and approves executed data packages and data summaries. • Authors validation summary reports.
Quality Assurance	• Reviews and approves validation protocols, data summaries, and reports. • Ensures that validation documents are in alignment with Moderna policies and regulatory requirements.

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3. Referenced Documents

Document #	Title
EP 2.2.1	Clarity and Degree of Opalescence of Liquids
EP 2.9.20	Particulate Contamination: Visible Particles
MQR-0002	Verification of SOP-0279, Osmolality of mRNA samples per USP <785>
MQR-0004	Verification of SOP-0288, pH Determination in the GMP Quality Control Laboratory
MQR-0050	Verification Report for SOP-0509, USP <788> Determination of Particulate Matter (Method 2)
MRA-C0000-GTM0018	Determination of Residual Ethanol in LNP Product
MRA-C0000-RTR0006	Acquisition of a GC Method for Residual Ethanol in a Lipid Nanoparticle Drug Product
SOP-0278	Appearance of Samples by Visual Inspection
SOP-0279	Osmolality of mRNA samples per USP <785>
SOP-0288	pH Determination in the GMP Quality Control Laboratory
SOP-0509	Determination of Particulate Matter
SOP-0950	Container Content for Injections: USP <697>
USP <467>	Residual Solvents
USP <631>	Color and Achromicity
USP <697>	Container Content for Injections
USP <785>	Osmolality and Osmolarity
USP <788>	Particulate Matter in Injections
USP <790>	Visible Particulates in Injections
USP <791>	pH
USP <1226>	Verification of Compendial Procedures

4. Materials and Equipment

4.1. Test Articles

The following table summarizes the types of representative test articles that were used to verify the various compendial test methods. The various test articles are representative verification materials and serve to properly verify method fitness for all sample types.

Sample Type	Appearance	pH	Osmolality	Container Content	Particulate Matter	Residual Solvents
mRNA		X				
DP		X	X		X	X

4.2. Materials and Equipment

Refer to the test method description in each SOP. Critical materials and equipment to ensure compendia suitability will be discussed by test method in subsequent sections.

5. Appearance

5.1. Scope

SOP-0278: Appearance of Samples by Visual Inspection, is a method to evaluate the appearance of samples (color, clarity, visible particulates) by visual inspection for cGMP release and stability testing of CX-024414 mRNA, mRNA-1273 LNP, mRNA-1273 DP, [redacted] in accordance with current USP <631>, EP 2.2.1, and EP 2.9.20.

Per current USP <1226>, verification requirements should be assessed based on the complexity of both the procedure and the material to which the procedure is applied. In subsequent sections, SOP-0278 will be compared against current USP <631>, EP 2.2.1, and EP 2.9.20 for suitability. The expected appearance of these products can be evaluated by this method.

5.2. Test Method Description

Visual inspection of samples is performed by visually assessing color, clarity, and the presence of visible particulates. Product is assessed in a portable manual inspection hood consisting of an appropriate light source and vertical, non-glare white and matte black panel backgrounds. The light source is capable of maintaining an intensity of illumination, at the viewing point, between 2000 and 3750 lux. The product is observed against both black and white backgrounds under full-spectrum lighting. Opalescence of the product is assessed against a water control (Ultrapure or equivalent RNase free), if applicable. The product is examined for the presence of visible particulates. The results of the color, clarity, and visible particulates assessments are reported as required per the associated specifications.

5.3. Verification

SOP-0278 is in harmony with the applicable current USP and EP chapters in that:

USP <631> Color and Achromicity: liquid samples are assessed in a liquid viewer against a white background under full-spectrum lighting. For products expected to be white to off-white, samples are assessed against the white background. For products expected to be colorless, samples are assessed against a water control. Water is considered to be achromic, or colorless, per USP <631>.

EP 2.2.1 Clarity and Degree of Opalescence of Liquids (Visual Method): liquid samples are assessed for opalescence in a liquid viewer against a black background under full-spectrum lighting and assessed against a water control contained in an identical test tube. A liquid is considered clear if its clarity is the same as that of water. Clarity is only assessed for CX-024414 mRNA.

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EP 2.9.20 Particulate Contamination: Visible Particles: Samples are assessed in an appropriate viewing station against both black and white backgrounds under full-spectrum lighting. As necessary, labels are removed from the outside of containers. The sample is gently swirled ensuring no air bubbles are introduced. The presence of any visible particles is recorded and characterized.

Expected appearance for each sample type is reported per the associated specification, as described in Table 1. All appearance attributes can be evaluated by SOP-0278 by comparing to either white background, black background, and/or water control.

Table 1: Appearance Specifications

Product Name	Expected Appearance (from specification)
CX-024414 mRNA	Clear, colorless solution, essentially free of visible particulates
mRNA-1273 LNP	White to off-white dispersion. May contain visible, white or translucent product-related particulates.
mRNA-1273 DP	White to off-white dispersion. May contain visible, white or translucent product-related particulates.
[redacted]	[redacted]

SOP-0278, Appearance of Samples by Visual Inspection, is considered fit for its intended use and verified to be consistent with USP <631>, EP 2.2.1, and EP 2.9.20.

6. pH

6.1. Scope

SOP-0288: pH Determination in the GMP Quality Control Laboratory, is a method used to determine the pH of cGMP release, and stability testing of CX-024414 mRNA, mRNA-1273 LNP, mRNA-1273 DP, [redacted] in accordance with current USP <791>.

SOP-0288 was verified using representative samples and results documented in method qualification report # MQR-0004. The verification parameters of Precision (Repeatability) and Precision (Intermediate) were assessed and met the acceptance criteria listed in the protocol. Evidence will be presented as to why this verification meets the requirements of USP <1226> for these additional products.

pH is a numerical scale used to specify the acidity or basicity of an aqueous solution. It is defined as the decimal logarithm of the reciprocal of the hydrogen ion activity, aH^+, in a solution.

6.2. Test Method Description

A suitable pH meter, such as the [redacted] or the [redacted], is utilized. These pH meters, including their associated electrodes, are operated in accordance with manufacturer recommendations and meet the instrument requirements listed in USP <791> including pH measurement resolution and ability to compensate for temperature.

The pH meter is calibrated (standardized) using commercially prepared, NIST traceable, standardization solutions each day of use using either a 2- or 3-point calibration, as appropriate to bracket the expected pH of samples to be tested. The standardization buffers will span no more than 4 pH units. The calibration must meet slope and offset acceptance criteria. The calibration is also verified using commercially prepared, NIST traceable, standardization solutions before measuring samples. The temperature adjusted pH value of the verification buffer reading must be [redacted] when compared to the label claim. The calibration, verification, and sample measurements are performed at room temperature.

6.3. Verification

SOP-0288 was successfully verified for precision (repeatability) and precision (intermediate). Refer to MQR-0004 for detailed results of the qualification testing utilizing representative test articles. Two independent pH meters were utilized, and both were successfully calibrated (standardized) and verified prior to executing precision. The range of the test articles utilized during this method verification is between [redacted] The range of the standards utilized during the method verification is [redacted]. The expected range of the new mRNA products are summarized in the table below. All expected pH values fall within the range that was verified during the method verification protocol. Refer to Table 2 for pH Specifications.

Table 2: pH Specifications

Product Name	Expected pH range (from specification)
CX-024414 mRNA	[redacted]
mRNA-1273 LNP	[redacted]
mRNA-1273 DP	[redacted]
[redacted]	[redacted]

SOP-0288, pH Determination in the GMP Quality Control Laboratory, is considered fit for its intended use and verified to be consistent with USP <791>.

7. Osmolality

7.1. Scope

SOP-0279: Osmolality of mRNA samples per USP <785>, is a method used to determine the osmolality of cGMP release and stability testing of mRNA-1273 LNP, mRNA-1273 DP, [redacted] in accordance with current USP <785> using freezing point depression.

SOP-0279 was verified using representative test articles and documented in method qualification report # MQR-0002. The verification parameters of Accuracy, Precision

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(Repeatability), and Precision (Intermediate) were assessed and met the acceptance criteria listed in the protocol. Evidence will be presented as to why this verification meets the requirements of USP <1226> for these additional products.

The Osmolality of a solution corresponds to the molality of an ideal solution containing non-dissociating solutes and is expressed in osmoles or milliosmoles per kilogram of solvent (Osm per Kg or mOsm per kg, respectively), a unit that is similar to the molality of the solution. Thus, osmolality is a measure of the osmotic pressure exerted by a solution across a semipermeable membrane. Like osmotic pressure, other colligative properties of a solution, such as vapor pressure lowering, boiling point elevation, and freezing point depression, are also directly related to the osmolality of the solution. Indeed, the osmolality of a solution is typically determined most accurately and conveniently by measuring freezing point depression (DTf): DTf = kf m where kf is the molal cryoscopic constant, which is a property of the solvent. For water, the value of kf is 1.860° per Osmol. That is, 1 Osmol of a solute added to 1 kg of water lowers the freezing point by 1.860°.

7.2. Test Method Description

Osmolality is determined by measuring the freezing point depression using a calibrated osmometer, such as the [redacted] The osmometer is calibrated by the manufacturer's instructions, in accordance with USP <785>. A 2- or 3- point calibration is performed each day of use, prior to testing samples, as appropriate to the range of samples to be analyzed. The calibration is verified with at least one calibration standard solution such that the osmolality of the standard solution lies within 50 mOsm/kg of the expected value of the sample to be analyzed or the center of the expected range of osmolality of the sample to be analyzed, with additional calibration standards verified for additional samples to be analyzed. Each calibration check must meet acceptance criteria. Samples are tested in triplicate and the average of the three readings reported as the final osmolality of the sample.

7.3 Verification

SOP-0279 was successfully verified for accuracy, precision (repeatability), and precision (intermediate) per SOP-0245. Refer to MQR-0002 for detailed results of the qualification testing. These parameters were evaluated on two micro-osmometers using various commercially prepared standard solutions and representative test articles. The range of the test articles utilized during this method verification is [redacted] The range of the standards utilized for this method verification is [redacted]. The expected range of the new mRNA products are summarized in Table 3. All expected osmolality values fall within the range that was verified by the method verification protocol.

Table 3: Osmolality Specifications

Product Name	Expected Osmolality range (from specification)
mRNA-1273 LNP	[redacted]
mRNA-1273 DP	
[redacted]	

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SOP-0279, Osmolality of mRNA samples per USP <785>, is considered fit for its intended use and is consistent with USP <785>.

8. Container Content

8.1 Scope

SOP-0950: Container Content for Injections: USP <697>, is a method used to determine the container content volume and labeled vialed fill size injections in multi-use containers of cGMP release testing of mRNA-1273 DP in accordance with current USP <697>. Per current USP <1226>, verification requirements should be assessed based on the complexity of both the procedure and the material to which the procedure is applied. In subsequent sections, SOP-0950 will be compared against current USP <697> for suitability. The expected container content of these products can be evaluated by this method.

The purpose of this test method is to determine the container content volume and labeled vialed fill size injections in multi-use containers of mRNA Drug Product samples. The scope of this procedure is to determine if sufficient excess volume is available for withdrawal according to the labeled vial quantity and dose(s) for mRNA drug products in multi-dose containers.

8.2 Test Method Description

Using an appropriately sized needle and syringe, the contents of the DP container are drawn up into a dry syringe at a volume to be measured as per the actual dose volume. The volume measured in an appropriately sized graduated cylinder is such that each syringe delivers not less than (NLT) the stated dose. This is repeated using separate syringe assemblies for as many full deliverable doses available.

8.3 Verification

SOP-0950 is in harmony with current USP <697> in that:

One multi-dose container is selected and the test method steps are per USP <697>. There are no critical instruments or reagents to assess. Container content of mRNA-1273 DP can be assessed using SOP-0950.

Table 4: Container Content Specification

Product Name	Expected Container Content (from specification)
mRNA-1273 DP	[redacted]

SOP-0950, Container Content for Injections: USP <697>, is considered fit for its intended use and is consistent with USP <697>.

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9. Particulate Matter

9.1. Scope

SOP-0509: Determination of Particulate Matter, is a method used to count particulate matter in cGMP release and stability testing of mRNA-1273 DP in accordance with current USP <788>. SOP-0509 was verified using representative test articles and documented in method qualification report # MQR-0050. The verification parameters of accuracy and precision (repeatability) were assessed and met the acceptance criteria listed in the protocol. Evidence will be presented as to why this verification meets the requirements of USP <1226> for these additional products.

SOP-0509 is performed in accordance with USP <788> (Method 2) "Particulate Matter in Injections, Method 2 Microscopic Particle Count Test" to assesses sub-visible (≥ 10 µm and ≥ 25 µm) particulate matter.

Particulate matter in injections and parenteral infusions consists of extraneous mobile undissolved particles, other than gas bubbles, unintentionally present in the solutions.

9.2. Test Method Description

Particulate matter is quantified by filtering samples and counting the particulates left on the filter membrane using a microscope in a particulate-free environment. The [redacted] or equivalent, is utilized and meets the requirements of USP <788> including 100X magnification, two suitable illuminators (one episcopic brightfield illuminator internal to the microscope and one focusable auxiliary illuminator adjusted to give reflected oblique illumination at an angle of 10° - 20°), and an ocular micrometer (circular diameter graticule integrated with the microscope). The filters utilized meet the requirements of USP <788> including nominal pore size. The particle sizes are estimated by comparing with the 10µm and 25µm reference circles on the graticule.

9.3. Verification

SOP-0509 was verified successfully for accuracy and precision (repeatability) per MQR-0050. These parameters were evaluated using representative test articles with all results meeting acceptance criteria. Particulate matter of ≥ 10 µm and ≥ 25 µm sizes were assessed, which corresponds to the size range of particulates quantified for mRNA-1273 DP, refer to Table 5, therefore no additional verification is required per USP <1226> for these additional products.

Table 5: Particulate Count Specification

Product Name	Expected Particulate Count (from specification)
mRNA-1273 DP	≥ 25 µm: ≤ 300 per container
	≥ 10 µm: ≤ 3000 per container

SOP-0509, Determination of Particulate Matter, is considered fit for its intended use and verified to be consistent with current USP <788>.

10. Residual Solvents

10.1. Scope

MRA-C0000-GTM0018: Determination of Residual Ethanol in LNP Product is a method used to detect residual ethanol in [redacted] in accordance with the current USP <467> using gas chromatography. MRA-C0000-GTM0018 was acquired by [redacted] per technical report # MRA-C0000-RTR0006 using representative test articles. During method acquisition, the method was assessed for accuracy and precision and met the acceptance criteria listed in the protocol.

[redacted] is in the process of performing a method validation specific to the [redacted] matrix to assess specificity, accuracy, precision, limit of detection (LOD), solution stability, and reproducibility. Results of this validation will be presented in a separate validation report. The initial verification will be discussed with respect to how it meets the requirements of USP <1226>. The below evidence supports the use of [redacted] MRA-C0000-GTM0018: Determination of Residual Ethanol in LNP Product for testing [redacted]

10.2. Test Method Description

The test method is a limit test for measuring the residual amount of Ethanol in the LNP Drug Product. According to USP <467>, Ethanol is a Class 3 residual solvent. The Permitted Daily Exposure for Ethanol is 50 mg per day. The limit of Ethanol is calculated suing Option 1 in the current ICH guideline, based on a 10 gram dose, thus the limit corresponds to [redacted]

Residual Ethanol in an [redacted] is analyzed using gas chromatography (GC) with a temperature gradient and headspace sampling and Flame Ionization Detection (FID), in accordance with USP <467> Residual Solvents, Water-soluble Articles, Headspace Operating Parameters 3, Procedure C. The Ethanol standard is prepared at the [redacted] level based on a [redacted] sample size, which is approximately [redacted]. The method utilizes the [redacted] Ethanol is eluted during an initial isocratic [redacted] minute hold at [redacted] The oven is then ramped at [redacted] per minute to [redacted] and held for an additional [redacted] minutes to clean out the system. The Ethanol residual solvent is detected using a flame ionization detector.

10.3. Verification

Analytical test method MRA-C0000-GTM0018 was acquired and verified by a Moderna-approved contract laboratory, [redacted] per MRA-C0000-RTR0006. Accuracy and precision were evaluated using representative test articles with all results meeting acceptance criteria. Results of this study demonstrate that the method is suitable for use as a limit test to determine the residual ethanol in LNP products.

Table 6: Residual Ethanol Specification

Product Name	Expected Residual Ethanol (from specification)
[redacted]	[redacted]

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MRA-C0000-GTM0018, Determination of Residual Ethanol in LNP Product, is considered fit for its intended use and verified to be consistent with USP <467>.

11. Conclusion

The verification of each compendial method is in accordance with Current USP <1226> Verification of Compendial Procedures, such that this report documents evidence of suitability under actual conditions of use. Each compendial method has been assessed as appropriate to the complexity of the procedure.

The compendial test methods discussed in this report have been found to be suitable for the testing of CX-024414 mRNA, mRNA-1273 Lipid Nanoparticle (LNP), mRNA-1273 Drug Product (DP), [redacted] samples.

12. Attachments

N/A

13. Revision History

Revision #	Effective Date	Change Details	Author
1.0	Refer to Veeva Header for Effective Date	New Document	[redacted]

Document Approvals

Approved Date: 05 Oct 2020

Task: Approval Task Verdict: Approve	[redacted] ([redacted]) Quality Control Approval 05-Oct-2020 16:52:13 GMT+0000

Task: QA Approval Task Verdict: Approve	[redacted] ([redacted]) Quality Assurance Approval 05-Oct-2020 17:05:06 GMT+0000