moderna Method Validation Report

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Method Validation Report: SOP-1032 Identity Confirmation of mRNA in a Lipid Nanoparticle by Sequencing Analysis

mRNA-1273 LNP, mRNA-1273 DP

1. Introduction

This report presents the method validation results of test method **SOP-1032**, Identity Confirmation of mRNA in a Lipid Nanoparticle by Sequencing Analysis, for mRNA-1273 LNP and DP. **SOP-1032** is categorized as an identity test and results are reported as conforms or does not conform. The validation was performed at the Moderna Quality Control Laboratory following method validation protocol **QC-MVP-0018 v1.0** and validation protocol addendum **QC-MVP-0021 v1.0** and is based on the ICH Q2(R1) Validation of Analytical Procedures: Text and Methodology.

SOP-1032, Identity Confirmation of mRNA in a Lipid Nanoparticle by Sequencing Analysis, is a method used to confirm the [redacted] of the open reading frame (ORF) of the mRNA LNP and DP by using Reverse transcription polymerase chain reaction (RT-PCR) to create complementary DNA template (cDNA) and analysis of cDNA by Sanger sequencing.

Method SOP-1032 was validated according to **QC-MVP-0018 v1.0**, using one lot of mRNA-1273 LNP and two lots of mRNA-1273 DP. The validation parameter of specify met the acceptance criteria listed in **QC-MVP-0018 v1.0**.

2. Referenced Documents

Document #	Title
ICH Q2(R1)	International Council for Harmonization, Validation of Analytical Procedures
FRM-0043	Read and Understand Training Form
FRM-0742	Assay Performance Worksheet- SOP-1032 Identity Confirmation of mRNA in Drug Product by Sequencing
SOP-1032	Identity Confirmation of mRNA in a Lipid Nanoparticle Drug Product by Sequencing Analysis
TR-9287	[redacted] - R+U - SOP-1032 and QC-MVP-0018
TR-10486	R + U SOP-1032 QC-MVP-0021 EXS
TR-10504	[redacted] - R+U QC-MVP-0021
QC-OTH-0199	QC-MVP-0021 Discrepancy 1
QC-MVP-0018	Method Validation Protocol SOP-1032 ID Confirmation of mRNA in a LNP by Sequencing
QC-MVP-0021	Method Validation Protocol SOP-1032 Identity Confirm of mRNA in a LNP by Seq Analysis - Addendum

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3. 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.

4. Documentation

3.1. All documentation, execution, and review of the work performed for this study was conducted under current Good Manufacturing Practices (cGMP) as required by Moderna standard operating procedures.

3.2. Draft **SOP-1032 v0.2** was followed for this testing. Assay information was documented on draft **FRM-0742**.

3.3. QC Analysts documented read and understand training on **SOP-1032 v0.2** and validation protocol **QC-MVP-0018 v1.0** and **QC-MVP-0021 v1.0** on **FRM-0043** prior to executing validation testing. Refer to [redacted] document **TR-9287, TR-10486,** and **TR-10504** for training record.

3.4. All relevant data collected during validation and formulae used for calculating validation characteristics will be included and discussed in this report.

5. Materials and Equipment

Draft **SOP-1032 v0.2** and draft **FRM-0742 v0.3** was followed for the validation testing.

5.1. Test Articles

Sample Description	Lot/Batch	RNA Concentration (mg/mL)	Summary of Analysis Document	Expected Length of RT-PCR Product (bp)
mRNA-1273 LNP[1]	5006820001	[redacted]	COA-0447	[redacted]
mRNA-1273 DP[2]	6006820001		COA-0448	
mRNA-1273 DP[2]	6006920001		COA-0449	

[1]mRNA-1273 Lipid Nanoparticle (LNP) is prepared as a liquid solution with a target mRNA concentration of [redacted] mg/mL in [redacted] mM sodium acetate, 20 mM Tris, 87 g/L Sucrose, pH 7.5 buffer.

[2]mRNA-1273 Drug Product (DP) is prepared as a liquid solution with a target mRNA concentration of approximately [redacted] mg/mL in [redacted] mM sodium acetate, 20 mM Tris, 87 g/L sucrose, pH 7.5 buffer.

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5.2. Reference Sequence of the [redacted] of the ORF of mRNA-1273

[redacted]

5.3. Test Primers for the [redacted] of the ORF of mRNA-1273

Primer Use	Primer Name	5'-Sequence-3'
RT-PCR	[redacted]	
RT-PCR/ Sequencing		
Sequencing		
Sequencing		

5.4. Materials and Equipment

Refer to the Materials and Equipment Section of **SOP-1032 v0.2**.

6. Validation Summary

6.1. Validation Parameters and Acceptance Criteria

Parameter	Acceptance Criteria	Results	Pass/Fail
Specificity (QC-MVP-0018)	Sequence matches 100% description of the [redacted] of the open reading frame with a [redacted]	The sequence generated for each of three lots of mRNA-1273 matches 100% description of the [redacted] of the open reading frame with [redacted]	Pass
Specificity (QC-MVP-0021)	The mRNA-1273 sample sequencing matches 100% description of the [redacted] of the open reading frame with a [redacted] The [redacted] non-target control reactions with mRNA-1273 primers does not generate sequence that 100% match the descriptions of the [redacted] of the open reading frame for mRNA-1273.	The sequence generated for mRNA-1273 lot 5006820001 matches 100% description of the [redacted] of the open reading frame with a [redacted] The sequence generated from [redacted] non-target control with mRNA-1273 primers did not generate sequence that was a 100% match the description of the [redacted] of the open reading frame for mRNA-1273.	Pass

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7. Validation Results

7.1. Specificity QC-MVP-0018 v1.0

Specificity is the ability to establish that the test method is specific to the product-related components.

Experimental Design:

To assess specificity, one lot of mRNA-1273 LNP (5006820001) and two lots of mRNA-1273 DP (6006820001 and 6006920001) were prepared and analyzed per SOP-1032 v0.2 following a [redacted] process.

Acceptance Criteria:

Sequence matches 100% description of the [redacted] of the open reading frame with a [redacted]

Results:

The sequence generated for each of three lots of mRNA-1273 matches 100% description of the [redacted]

7.2. Specificity QC-MVP-0021 v1.0

Specificity is the ability to establish that the test method is specific to the product-related components.

Experimental Design:

To assess specificity, one lot of mRNA-1273 LNP lot 5006820001 and one lot of non-target mRNA control [redacted] lot MTDS18026 were prepared and analyzed per SOP-1032 v0.2 using primer designed for mRNA-1273 following the [redacted] process.

Acceptance Criteria:

The mRNA-1273 sample sequencing matches 100% description of the [redacted] of the open reading frame with a [redacted]

The [redacted] non-target control reactions with mRNA-1273 primers does not generate sequence that 100% match the descriptions of the [redacted] of the open reading frame for mRNA-1273.

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Results:

The sequence generated for mRNA-1273 LNP lot 5006820001 matched 100% description of the [redacted] of the open reading frame with a [redacted]

The sequence generated from [redacted] non-target mRNA control with mRNA-1273 primers did not generate sequence that was a 100% match the description of the [redacted] of the open reading frame for mRNA-1273.

8. Discrepancies

There was one discrepancy associated with the execution of this validation (**QC-OTH-0199 v1.0**). This discrepancy was linked to operator error and the assay run was repeated successfully. This discrepancy has no impact on this validation.

9. Conclusions

Analytical test method **SOP-1032** passes the acceptance criteria for all validation parameter outlined in protocols **QC-MVP-0018 v1.0** and **QC-MVP-0021 v1.0** for Specificity.

Analytical test method **SOP-1032** is considered validation for testing mRNA-1273 LNP and DP. A verified data summary for the validation assays is attached (**Attachment 1**).

10. Attachments

Attachment 1: QC-MVR-0018 Verified Data Summary [redacted]

Attachment 2: ARN-20-00333-012 for QC-MVP-0018 [redacted]

Attachment 3: ANR-20-0401-035 for QC-MVP-0021 Invalid [redacted]

Attachment 4: ARN-20-00419-025 for QC-MVP-0021 Repeated [redacted]

11. Revision History

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

Document Approvals

Approved Date: 05 Oct 2020

Task: Approval Task Verdict: Approve	[redacted]
Task: Approval Task Verdict: Approve	[redacted]
Task: QA Approval Task Verdict: Approve	[redacted]