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Method Validation Report of SOP-1000: % Encapsulation Efficiency by ▮▮▮▮▮▮	
mRNA-1273 LNP, mRNA-1273 DP	

1. Introduction

This report presents the method validation results of test method SOP-1000 for mRNA-1273 Lipid Nanoparticle (LNP) and mRNA-1273 Drug Product (DP). The validation was performed at the Moderna Quality Control (QC) Laboratory following method validation protocol QC-MVP-0009 and in in accordance with the ICH Q2(R1) Guideline for Validation of Analytical Procedures.

SOP-1000, % Encapsulation Efficiency ▮▮▮▮▮▮▮▮, is a method to determine free mRNA and the % Encapsulation Efficiency (%EE) in mRNA LNP and DP samples ▮▮▮▮▮▮▮▮ ▮▮▮▮▮▮▮▮▮.

Analytical test method SOP-1000 was validated according to protocol QC-MVP-0009 using CX-024414 mRNA, mRNA-1273 LNP and mRNA-1273 DP. The validation parameters of specificity, linearity, accuracy, precision (repeatability, intermediate precision), range, quantitation limit, and robustness were evaluated and met the acceptance criteria listed in the protocol.

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.

3. 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 analytical method SOP-1000 (version 0.3) was followed for this testing. Assay information was documented on draft FRM-0735 (version 0.5).

3.3. QC Analysts documented read and understand training on analytical method SOP-1000 and validation protocol QC-MVP-0009 prior to executing validation testing. Refer to ▮▮▮▮ documents TR-9538 and TR-9539.

3.4. Protocol discrepancies were documented in the ▮▮▮▮▮▮ digital quality system and are referenced in this validation report. One discrepancy occurred and documented in QC-OTH-0169.

3.5. All relevant data collected during validation testing and formulae used for calculating validation characteristics was peer reviewed, data verified, and included as attachments to this validation report.

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4. Materials and Equipment

4.1. Test Articles

Sample Description	Lot/Batch	RNA Concentration (mg/mL)	SoA or CoA ███ Document #
███████	AMPDP-20062		N/A
CX-024414 mRNA	MTDS20002		DSAD-SOA-0254
mRNA-1273 LNP	5006820001		COA-0447
mRNA-1273 DP	DHM-47504		DPAD-00817
mRNA-1273 DP	6006820001		COA-0448

4.2. Materials and Equipment

Refer to the Materials and Equipment Section of SOP-1000 (version 0.3).

5. Validation Summary

5.1. Validation Parameters and Acceptance Criteria

Parameter	Acceptance Criteria	Results	Pass / Fail
System Suitability	Report system suitability results as outlined in analytical test method SOP-1000.	System suitability per SOP-1000 version 0.3 passed acceptance criteria. Refer to Attachment I for results.	Pass
Specificity	███████ interference for detecting free mRNA is ███ EE.	███ matrix interference for detecting free mRNA is ██ EE. Detected Free mRNA = ████████	Pass
Linearity	The correlation coefficient (R²) of the Linear Regression of the spiked (experimental) Free mRNA and theoretical free mRNA must be ████	$R^2 =$ ██████	Pass

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Parameter	Acceptance Criteria	Results	Pass / Fail
Accuracy	The %EE % Recovery at each level must be ▮▮▮ of the theoretical %EE for levels ▮▮ and ▮▮▮ for levels ▮▮[1] The %RSD of the triplicate %EE results at each level must be ▮▮▮	**Mean %EE %Recovery:** ▮▮▮▮▮ **The %RSD of the triplicate %EE results:** ▮▮▮▮▮	Pass (levels 1-8)
Precision (Repeatability)	The %RSD of the % EE results ▮▮▮ for each test article must be ▮▮▮	**Analyst 1 %RSD:** mRNA-1273 LNP Lot 5006820001 = ▮▮▮ mRNA-1273 DP lot DHM-47504 = ▮▮▮ mRNA-1273 DP Lot 6006820001 = ▮▮▮	Pass
Precision (Intermediate)	The %RSD of % EE results for Analyst 2 ▮▮ must be ▮▮ ▮▮▮ for each test article. The overall %RSD % EE results for both analysts ▮▮▮ must be ▮▮▮ for each test article. The Absolute Difference in the % EE results between analysts must be ▮▮	**Analyst 2 %RSD:** mRNA-1273 LNP Lot 5006820001 = ▮▮▮ mRNA-1273 DP lot DHM-47504 = ▮▮▮ mRNA-1273 DP Lot 6006820001 = ▮▮▮	Pass

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[1] Updated linearity levels and criteria per discrepancy QC-OTH-0169

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Parameter	Acceptance Criteria	Results	Pass / Fail
Precision (Intermediate)		**Analyst 1 and 2 %RSD:** mRNA-1273 LNP Lot 5006820001 = ▮ mRNA-1273 DP lot DHM-47504 = ▮ mRNA-1273 DP Lot 6006820001 = ▮ **The Absolute Difference in the % EE results:** mRNA-1273 LNP Lot 5006820001 = ▮ mRNA-1273 DP lot DHM-47504 = ▮ mRNA-1273 DP Lot 6006820001 = ▮	
Range	If the validation target expectations for linearity, precision, and accuracy are met, this demonstrates that the range is suitable.	**Validated Range:** ▮ EE	Pass
Quantitation Limit (QL)	QL of the assay is the lowest level of %EE that meets the acceptance criteria in the accuracy and linearity sections.	**Validated QL = ▮ EE**	Pass
Robustness	Intermediate precision criteria are met.	Intermediate precision criteria were met.	Pass

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6. Validation Results

6.1. System Suitability

Experimental Design:

System suitability was evaluated per SOP-1000 for all validation testing.
Acceptance Criteria:

Report system suitability results as outlined in analytical test method SOP-1000.

Results will be assessed during the validation and any necessary updates to draft versions of SOP-1000 and FRM-0735 will be made prior to the effective versions.

Results:

System suitability passed the acceptance criteria listed in SOP-1000. No further updates to the system suitability criteria will be made based on results of this validation.

6.2. Specificity

Experimental Design:

- Three separate preparation of ▮▮▮▮▮ lot AMPDP-20062 were made by spiking ▮▮▮▮▮▮▮▮▮▮▮ working solution then tested following SOP-1000

Data Analysis:

The % Encapsulation Efficiency result for each ▮▮▮▮▮ preparation tested was calculated.

Acceptance Criteria:

▮▮▮▮▮ does not show any matrix interference for detecting free mRNA. This is demonstrated by obtaining a result of ≤ ▮ Encapsulation Efficiency (EE) for all spiked preparations.

Results:

The free mRNA detected for all preparations was ▮▮▮▮ and %EE was ▮▮ for all preparations. Results met the acceptance criteria, demonstrating that the ▮▮▮▮ matrix does not show any matrix interference for detecting free mRNA. Refer to Table 1 for results.

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Table 1. Specificity Results

███████ Preparation	Free mRNA (mg)	%EE
1	███████	███████
2	███████	███████
3	███████	███████

6.3.   Linearity

<u>Experimental Design (Linearity):</u>

Linearity was evaluated for mRNA-1273 DP over a range of █████████ Encapsulation ██ corresponds to █████ Encapsulation Efficiency (upper limit). █████ corresponds to ███ Encapsulation Efficiency (lower limit). Linearity levels were updated (due to initial failures), and rationale documented in QC-MVP-0009 Discrepancy # 1 █████ document # QC-OTH-0169).

- Free mRNA (mg) for sample mRNA-1273 DP (lot DHM-47504) was measured (in triplicate) per SOP-1000. This will be █████
- Linearity levels ███ were prepared per instructions in protocol QC-MVP-0009 Attachment 1, and some levels subsequently repeated per discrepancy QC-OTH-0169 (updated levels).
- Samples were tested per SOP-1000.

Table 2: Linearity Execution Details

<u>Data Analysis:</u>

- The free mRNA results (mg) and mean %EE recovery for each preparation at each level were calculated.
- The %RSD of the triplicate measured % EE results at each level was calculated.
- A linear regression analysis of the experimental Δ measured free mRNA values (mg) against the free mRNA target values (mg) was performed.

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- The regression line was plotted, and a residual plot produced.
- The correlation coefficient (R^2), slope, and Y-intercept were calculated and recorded.

Experimental Design (Accuracy):

Per ICH guidelines, accuracy is evaluated on samples spiked with known amounts of analyte over a minimum of 3 concentrations levels with 3 replicates at each level covering the specified range. Accuracy may also be inferred once precision linearity and specificity have been established. Since the linearity experiment had ▮▮▮▮ with triplicate preparations at each level, separate experiments were not needed to evaluate the accuracy of the test method.

Acceptance Criteria:

Linearity

The correlation coefficient (R^2) of the Linear regression must be ▮▮▮▮

Accuracy

The %RSD of the triplicate measured % EE results at each level must be ▮▮▮▮.

The %EE %Recovery at each level must be ▮▮▮▮▮ of the theoretical concentration (original criteria in protocol; superseded).

The %EE % Recovery at each level must be ▮▮▮▮▮ of the theoretical %EE for ▮▮▮▮ and ▮▮▮▮ for levels ▮▮ (updated criteria per discrepancy #1, #QC-OTH-0169).

Results:

Results for linearity and accuracy met the acceptance criteria for levels ▮▮ corresponding to ▮▮▮▮ EE. Results met the acceptance criteria for %EE %RSD and %EE Recovery and are presented in Table 3.

Level ▮ which corresponds to ▮▮▮ EE did not meet the accuracy acceptance criteria for %EE %RSD or %EE %Recovery and thus was not included when plotting the linear regression. Refer to Figure 1 for the linear regression plot. The R^2 for levels ▮▮ is ▮▮▮▮

A discrepancy occurred during the execution of the linearity / accuracy experiments and documented in QC-MVP-0009 discrepancy #1, ▮▮▮▮ document QC-OTH-0169. Spiked Free mRNA (mg) values were listed incorrectly in protocol QC-MVP-0009 section 5.4 tables 1 and 2. %EE % recovery values did not meet the acceptance criteria for levels ▮▮▮▮ The correct free mRNA (mg) values were updated, linearity experiment design modified, a new level added (▮▮▮▮, and acceptance criteria widened for linearity levels ▮▮▮▮ and ▮▮▮. Experiments were re-executed following the updates to the linearity spike strategy described in the discrepancy document. The complete dataset, including initial results, can be found in Attachments 2 and 3. The final dataset is listed in Table 3.

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Table 3: Linearity and Accuracy Data

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Figure 1. Linearity Regression
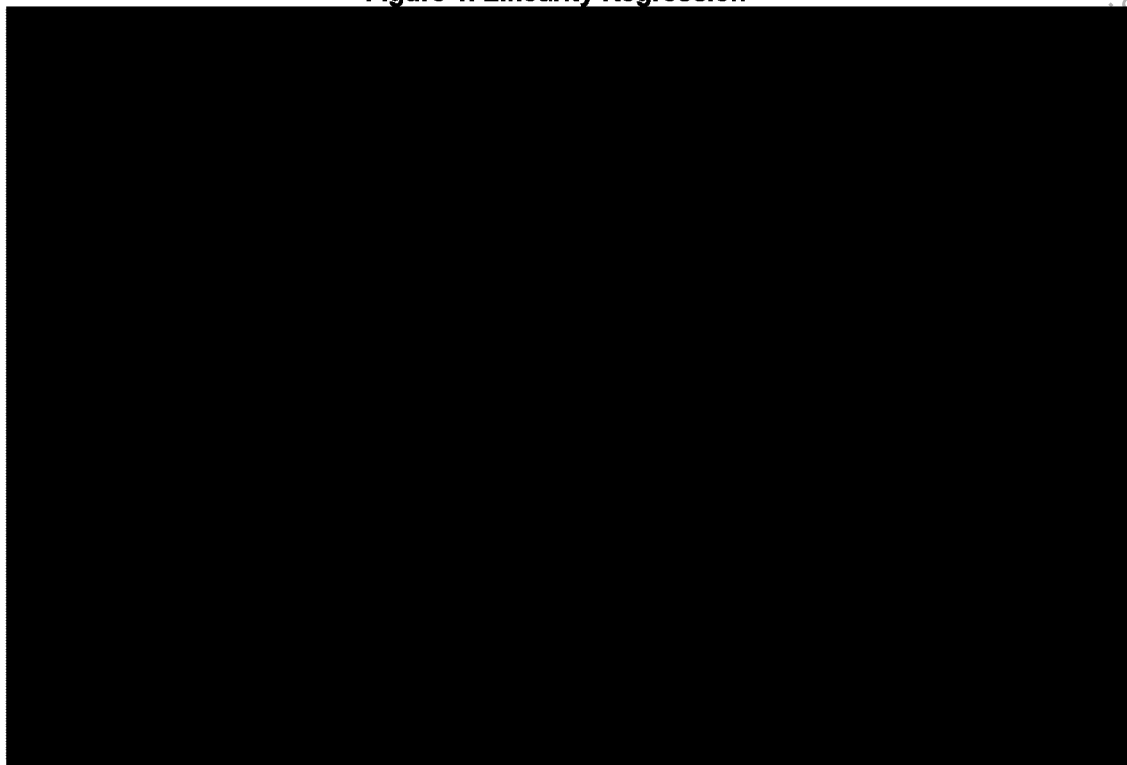
6.4. Precision (Repeatability)

Experimental Design:

Analyst 1 made ▉ sample preparations of each lot of mRNA-1273 LNP and DP and analyzed each preparation once per SOP-1000. Analyst 1 used the ▉▉▉▉▉▉▉ brands for sample analysis. Refer to Table 4 for details.

Table 4: Precision Study Details

Sample	Batch	Number of Sample Preparations
mRNA-1273 LNP	5006820001	▉▉▉
mRNA-1273 DP	DHM-47504	▉▉▉
mRNA-1273 DP	6006920001	▉▉▉

Data Analysis:

The mean % EE results ▉▉▉ and %RSD for each test article were calculated.

Acceptance Criteria:

The %RSD of the % EE results ▉▉▉ for each test article for Analyst 1 must be ▉▉▉▉▉

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Results:

Precision data for analyst 1 met the %RSD criteria for all samples assessed, demonstrating acceptable intra-assay precision. Results are presented in Table 5.

Table 5. Precision (Repeatability) Analyst 1 Results

Sample	%EE	Mean %EE	%RSD
mRNA-1273 LNP Lot 5006820001	███	███	███
mRNA-1273 DP Lot DHM-47504	███	███	███
mRNA-1273 DP Lot 6006820001	███	███	███

6.5.   Precision (Intermediate)

Experimental Design:

On a separate day, a second analyst (Analyst 2) repeated Precision (section 6.5) using the same lots of mRNA-1273 LNP and DP test articles.

Analyst 2 tested the samples using a different cuvette brand that was used by Analyst 1 (Brand Tech).

Data Analysis:

The mean % EE results ███ and %RSD for each test article were calculated for Analyst 2.

The mean % EE results (███ and %RSD for each test article were calculated for Analyst 1 and 2 combined data.

The absolute difference of the mean %EE results was determined between Analyst 1 and Analyst 2 for each test article.

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Acceptance Criteria:

The %RSD of the % EE results for Analyst 2 (▮▮▮) must be ▮▮▮▮ for each test article analyzed.

The overall %RSD of the % EE results for both analysts (▮▮▮) must be ▮▮▮▮ for each test article

The Absolute Difference in the % EE results between analysts must be ▮▮▮

Results:

Precision data for analyst 2 met the %RSD criteria for all samples assessed.

Precision data for analyst 1 and 2 met the %RSD and absolute difference criteria for all samples assessed, demonstrating acceptable inter-assay precision.

Results are presented in Tables 6-7.

Table 6. Precision (Intermediate) Analyst 2 Results

Sample	%EE	Mean %EE	%RSD
mRNA-1273 LNP Lot 5006820001	███	███	███
mRNA-1273 DP Lot DHM-47504	███	███	███
mRNA-1273 DP Lot 6006820001	███	███	███

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Table 7. Precision A1 vs. A2 Results

Sample	A1 + A2 Mean (n=12)	%RSD	Absolute Difference in mean %EE Results
mRNA-1273 LNP Lot 5006820001	███	███	███
mRNA-1273 DP Lot DHM-47504	███	███	███
mRNA-1273 DP Lot 6006820001	███	███	███

6.6.   Range

If the validation target expectations for linearity, precision, and accuracy are met, this demonstrates that the range is suitable.

The validated range was determined to be ▮▮▮▮ EE.

6.7.   Quantitation Limit

Experimental Design:

The QL was determined experimentally based on the data generated for linearity and accuracy. The lowest level meeting the precision requirement from the linearity section (% RSD) and accuracy (% recovery) acceptance criteria will be set as the QL of the method.

Data Analysis:

Accuracy (% recovery), linearity ▮▮▮▮, and precision (%RSD) will be reported for the lowest linearity level.

Acceptance Criteria:

QL of the assay is the lowest %EE tested in the accuracy and linearity sections that meets the following acceptance criteria:

Precision (from the accuracy section): The %RSD for the %EE results for the preparations of each level must be ▮▮▮▮

Accuracy: The %recovery at the lowest %EE level must be within ▮▮▮▮

Results:

The QL was determined to be ▮▮▮ The results at this level are as follows:

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6.8.   Robustness

Robustness was assessed in this validation protocol by varying assay conditions that may occur under normal usage in QC, while intentionally varied assay parameters were evaluated during assay development. Intermediate Precision data was used to support the robustness assessment.

Acceptance Criteria:

Intermediate precision criteria are met.

Results:

Intermediate Precision criteria were met.

7. Discrepancies

One discrepancy occurred during the execution of this validation, is discussed in section 6.3 of this report, and documented in █████ document # QC-OTH-0169.

8. Conclusions

Analytical test method SOP-1000 met the acceptance criteria for validation parameters described in protocol QC-MVP-0009: specificity, linearity, accuracy, precision (repeatability, intermediate precision), range, QL, and robustness.

Analytical test method SOP-1000 is considered validated for testing mRNA-1273 LNP and mRNA-1273 DP samples. The linear range has been determined to be ████████EE, while the QL was determined to be ████.

A verified data summary for the validation experiments is attached, along with the peer-reviewed source raw data packages. Refer to Attachments 1 – 3.

9. Referenced Documents

Document #	Title
ICH Q2(R1)	International Council for Harmonization, Validation of Analytical Procedures
FRM-0735	SOP-1000 Assay Performance Worksheet
SOP-1000	% Encapsulation ████████
TR-9538	████████ - R+U - SOP-1000 v0.3 and QC-MVP-0009 v. 1.0
TR-9539	████████ - - R+U - SOP-1000 v0.3 and QC-MVP-0009 v. 1.0
QC-MVP-0009	Method Validation Protocol of SOP-1000: % Encapsulation ██ ████████
QC-OTH-0169	Method Validation Protocol Discrepancy for QC-MVP-0009: Discrepancy #1

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10. Attachments

Attachment 1: QC-MVP-0009 Data Portfolio ████

Attachment 2: QC-MVP-0009 Verified Excel Data (████

Attachment 3: QC-MVP-0009 Excel Data ██████

11. Revision History

Revision #	Effective Date	Change Details	Author
1.0	Refer to ████ Header for Effective Date	New Document	████████

Document Approvals
Approved Date: 05 Oct 2020

Task: Approval Task Verdict: Approve	▮▮▮▮▮▮▮▮▮▮ Quality Control Approval 05-Oct-2020 19:38:40 GMT+0000

Task: QA Approval Task Verdict: Approve	▮▮▮▮▮▮▮▮▮▮ Quality Assurance Approval 05-Oct-2020 21:45:57 GMT+0000