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Method Validation Report of SOP-0998: Determination of Particle Size Distribution and Polydispersity by Dynamic Light Scattering

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

1. Introduction

This report presents the method validation results of test method of test method SOP-0998 for mRNA-1273 Lipid Nanoparticle (LNP), mRNA-1273 Drug Product (DP), [redacted]. The validation was performed at the Moderna Quality Control (QC) Laboratory following method validation protocol QC-MVP-0011: Method Validation Report of SOP-0998: Determination of Particle Size Distribution and Polydispersity by Dynamic Light Scattering, and in accordance with the ICH Q2(R1) Guideline for Validation of Analytical Procedures.

SOP-0998, Determination of Particle Size Distribution and Polydispersity by Dynamic Light Scattering (DLS), is a method to determine the hydrodynamic diameter (average size) and size distribution of Lipid Nanoparticle (LNP) formulations. This is a quantitative determination assay for samples using particle size standards as system suitability checks.

Method SOP-0998 was validated according to protocol QC-MVP-0011 using mRNA-1273 LNP, mRNA-1273 DP, [redacted]. The validation parameters of specificity, accuracy, precision (repeatability, intermediate precision), range, and robustness were assessed 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-0998** (version 0.2) was followed for this testing. Assay information was documented on draft **FRM-0728** (version 0.2).

3.3. QC Analysts documented read and understand training on analytical method **SOP-0998** and validation protocol **QC-MVP-0011** prior to executing validation testing. Refer to Veeva documents **TR-9540** and **TR-9541** for the training records.

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

4. Materials and Equipment

4.1. Test Articles

Sample Description	Lot/Batch	RNA Concentration (mg/mL)	Target Size
mRNA-1273 LNP	5006820001	[redacted]	
[redacted]	AMPDP-20062		
mRNA-1273 DP	6006820001		
mRNA-1273 DP	6006920001		

4.2. Materials and Equipment

Refer to the Materials and Equipment Section of SOP-0998 (version 0.2).

5. Validation Summary

5.1. Validation Acceptance Criteria and Results

Parameter	Acceptance Criteria	Results	Pass / Fail
System Suitability	Report system suitability results as outlined in analytical test method SOP-0998.	System suitability per SOP-0998 version 0.2 passed acceptance criteria. Refer to Attachment I for results.	Pass
Specificity	The average normalized intensity from the blank PBS sample must be [redacted]	The average normalized intensity from the blank PBS sample result was [redacted]	Pass

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Parameter	Acceptance Criteria	Results	Pass / Fail
Accuracy	For each size standard, the %RSD of the triplicate diameter (nm) readings must be [redacted] For each size standard, the difference between CoA and the average measured particle size value must be [redacted] PDI for all measurements [redacted]	**Particle Size (nm) %RSD:** [redacted] **Std Difference against CoA:** [redacted] **PDI for all measurements:** [redacted]	Pass
Precision (Repeatability)	The particle size %RSD of the 6 replicate values for each test article must be [redacted] Report the individual and mean of the polydispersity index (PdI) of the 6 replicate values for each test article	**Analyst 1 Particle Size (nm) %RSD:** mRNA-1273 LNP Lot 5006820001 — %RSD: [redacted] mRNA-1273 DP Lot 6006820001 mRNA-1273 DP Lot 6006920001 [redacted] Lot AMPDP-200062 **Analyst 1 PDI values:** **mRNA-1273 LNP Lot 5006820001** — Preps 1–6: [redacted]	Pass

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<table>
<tr><th>Parameter</th><th>Acceptance Criteria</th><th>Results</th><th>Pass / Fail</th></tr>
<tr><td>Precision (Repeatability)</td><td></td><td>
<table>
<tr><th>Sample</th><th>Prep</th><th>PdI</th><th>Average PdI</th></tr>
<tr><td rowspan="6">mRNA-1273 DP Lot 6006820001</td><td>1</td><td rowspan="6" colspan="2">[redacted]</td></tr>
<tr><td>2</td></tr>
<tr><td>3</td></tr>
<tr><td>4</td></tr>
<tr><td>5</td></tr>
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<tr><th>Sample</th><th>Prep</th><th>PdI</th><th>Average PdI</th></tr>
<tr><td rowspan="6">[redacted] Lot AMPDP-200062</td><td>1</td><td rowspan="6" colspan="2">[redacted]</td></tr>
<tr><td>2</td></tr>
<tr><td>3</td></tr>
<tr><td>4</td></tr>
<tr><td>5</td></tr>
<tr><td>6</td></tr>
</table>
</td><td></td></tr>
<tr><td>Precision (Intermediate)</td><td>For Analyst 2, the particle size %RSD of the 6 replicate values for each test article must be [redacted]
For Analyst 1 and 2, the particle size %RSD of sample replicates (n=12) for each test article must be [redacted]</td><td></td><td>Pass</td></tr>
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mRNA-1273 LNP, mRNA-1273 DP, [redacted]

Parameter	Acceptance Criteria	Results	Pass / Fail
Precision (Intermediate)	The % Difference of mean particle size between Analyst 1 and 2 [redacted] For Analyst 1 and 2, report the individual and mean of the polydispersity index (PdI) of the sample replicate (n=12) values for each test article.	**Analyst 2 Particle Size (nm) %RSD:** Sample: mRNA-1273 LNP Lot 5006820001; mRNA-1273 DP Lot 6006820001; mRNA-1273 DP Lot 6006920001; [redacted] Lot AMPDP-200062 %RSD: [redacted] **Analyst 1 and 2 Particle Size (nm) %RSD:** Sample: mRNA-1273 LNP Lot 5006820001; mRNA-1273 DP Lot 6006820001; mRNA-1273 DP Lot 6006920001; [redacted] Lot AMPDP-200062 %RSD: [redacted] **% Difference of mean particle size (nm) between Analyst 1 and 2:** Sample: mRNA-1273 LNP Lot 5006820001; mRNA-1273 DP Lot 6006820001; mRNA-1273 DP Lot 6006920001; [redacted] Lot AMPDP-200062 %Diff: [redacted]	

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

Parameter	Acceptance Criteria	Results	Pass / Fail
Precision (Intermediate)		**Analyst 2 and combined PDI values:**	

Sample	Prep	Pdl	Average Pdl
mRNA-1273 LNP Lot 5006820001	1	[redacted]	
	2		
	3		
	4		
	5		
	6		
A1 and A2 (n=12)			[redacted]

Sample	Prep	Pdl	Average Pdl
mRNA-1273 DP Lot 6006820001	1	[redacted]	
	2		
	3		
	4		
	5		
	6		
A1 and A2 (n=12)			[redacted]

Sample	Prep	Pdl	Average Pdl
mRNA-1273 DP Lot 6006920001	1	[redacted]	
	2		
	3		
	4		
	5		
	6		
A1 and A2 (n=12)			[redacted]

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

Parameter	Acceptance Criteria	Results	Pass / Fail
		Sample: [redacted] Lot AMPDP-200062; Preps 1–6: Pdl [redacted], Average Pdl [redacted]; A1 and A2 (n=12): [redacted]	
Range	If the qualification target expectations for precision and accuracy are met, report the range of values over which the method is linear with an acceptable degree of precision and accuracy.	Range: [redacted]	Pass
Robustness	Intermediate precision criteria are met.	Intermediate precision criteria were met.	Pass

6. Validation Results

6.1. Specificity and Accuracy

Experimental Design:

Triplicate preparations each of the particle size standards ([redacted]) and a blank PBS sample were made and analyzed per SOP-0998.

Specificity Data Analysis

The Average Normalized Intensity (kCnts/s) results of the blank PBS sample was compared with the results from the [redacted] size standard measurements.

Accuracy Data Analysis

A plot was generated for the measured particle size (in nm) versus nominal (theoretical) mean diameter values.

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

The average particle size for each triplicate reading for the [redacted] particle size standards was calculated. For a size standard to be included in the accuracy and range determination, the %RSD must be [redacted] and the mean diameter must be [redacted] of the mean diameter reported in the Certificate of Analysis (CoA).

The difference between average measured particle size and the size value reported on the CoA for each particle size standard was calculated and reported.

Specificity Acceptance Criteria

The average normalized intensity from the blank PBS sample must be [redacted]

Accuracy Acceptance Criteria:

For each size standard, the %RSD of the triplicate diameter (nm) readings must be [redacted]

For each size standard, the difference between CoA and the average measured particle size value must be [redacted]

Specificity Results

The average normalized intensity from the blank PBS sample result was [redacted]. Results are presented in Table 1. The results demonstrate that there is no interference due to the blank PBS sample.

Table 1. Specificity Results

Sample	Average Normalized Intensity (kCnts/s)
[redacted]	

Accuracy Results

For each size standard, the %RSD of the triplicate diameter (nm) reading was [redacted] for all size standards assessed [redacted].

For each size standard, the average measured particle size value was within [redacted] of the CoA value. Results are presented in Table 2. Figure 1 is a plot the measured particle size (in nm) versus nominal (theoretical) mean diameter values.

The results demonstrate accuracy across the range of [redacted] particle size.

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

Table 2. Accuracy Results

Theoretical Particle Size (nm)	CoA Value (nm)	Standard Prep	Measured Particle Size (nm)	PDI	Mean Measured Particle Size (nm)	Difference of Particle Size value (nm) vs. CoA	Particle Size %RSD
[redacted]		1	[redacted]				
		2					
		3					
		1					
		2					
		3					
		1					
		2					
		3					

Figure 1. Measured particle size (in nm) versus nominal mean diameter values

[redacted]

6.2. Precision (Repeatability and Intermediate Precision)

Repeatability Experimental Design

Analyst 1 made six preparations (n=6) each of mRNA-1273 LNP, mRNA-1273 DP, and [redacted] test articles at the target final concentration of the method per SOP-0998.

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Intermediate Precision Experimental Design

A second analyst (Analyst 2) repeated the repeatability precision assay on a separate day and using different preparations of standard and sample dispersants than Analyst 1. The same lots of test articles were used to perform intermediate precision assay.

Precision data from Analyst 1 and Analyst 2 were combined for n=12.

Data Analysis

The particle size and polydispersity index (PdI) for each replicate was reported for each analyst

The %RSD of the 6 replicate values for each test article was calculated for each analyst.

The %RSD of the 12 replicate values for each test article was calculated for analyst 1 and 2.

Acceptance Criteria

For each analyst, the particle size %RSD of the 6 replicate values for each test article must be [redacted]

For Analyst 1 and 2, the particle size %RSD of sample replicates (n=12) and % difference of mean particle size for each test article must be [redacted]

For Analyst 1 and 2, report the individual and mean of the sample polydispersity index (PdI) of the sample replicate (n=12) values for each test article.

Results

Results passed all repeatability precision and intermediate precision acceptance criteria for %RSD and %Difference, demonstrating intra-assay and inter-assay precision. Results are presented in Tables 3 –[redacted]

mRNA-1273 LNP mean (n=12) particle size result was [redacted] and %difference of [redacted]

mRNA-1273 DP Lot 6006820001 mean (n=12) particle size result was [redacted] and %difference of [redacted]

mRNA-1273 DP Lot 6006920001 mean (n=12) particle size result was [redacted] and %difference of [redacted]

[redacted] mean (n=12) particle size result was [redacted] and %difference of [redacted]

Table 3. Precision Results for mRNA-1273 LNP Lot 5006820001

mRNA-1273 LNP Lot 5006820001	Prep	Particle Size (nm)	PdI	Average Particle Size	Average PdI	%RSD Particle Size	% Difference
Analyst 1	1	[redacted]					
	2						
	3						
	4						
	5						
	6						
Analyst 2	1						
	2						
	3						
	4						
	5						
	6						
Analyst 1&2							

Table 4. Precision Results for mRNA-1273 DP Lot 6006820001

mRNA-1273 DP Lot 6006820001	Prep	Particle Size	PdI	Average Particle Size	Average PdI	%RSD Particle Size	% Difference
Analyst 1	1	[redacted]					
	2						
	3						
	4						
	5						
	6						
Analyst 2	1						
	2						
	3						
	4						
	5						
	6						
Analyst 1&2							

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

Table 5. Precision Results for mRNA-1273 DP Lot 6006920001

mRNA-1273 DP Lot 6006920001	Prep	Particle Size	PdI	Average Particle Size	Average PdI	%RSD Particle Size	% Difference
Analyst 1	1	[redacted]					
	2						
	3						
	4						
	5						
	6						
Analyst 2	1						
	2						
	3						
	4						
	5						
	6						
Analyst 1&2							

[redacted]

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

6.3. Range

The validation target expectations for accuracy and precision were met.

The range at which the method is linear with an acceptable degree of precision and accuracy is [redacted] particle size.

6.4. Robustness

Robustness were 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. The parameters varied for this validation were: analyst, day, and standard and sample dispersants preparations. 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

No discrepancies occurred during this validation execution.

8. Conclusion

Analytical test method SOP-0998 passed the acceptance criteria for validation parameters in protocol QC-MVP-0011: specificity, accuracy, precision (repeatability, intermediate precision), range and robustness

Analytical test method SOP-0998 is considered validated for particle size distribution and polydispersity by DLS testing of mRNA-1273 LNP, mRNA-1273 DP, [redacted] samples.

The validated particle size range was determined to be [redacted]

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

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

9. Referenced Documents

Document #	Title
ICH Q2(R1)	International Council for Harmonization, Validation of Analytical Procedures
FRM-0728	SOP-0998 Assay Performance Worksheet
TR-9540	[redacted]- R+U - SOP-0998 v0.2 and QC-MVP-0011 v.1.0
TR-9541	[redacted]- R+U - SOP-0998 v0.2 and QC-MVP-0011 v.1.0
SOP-0998	Determination of Particle Size Distribution by Dynamic Light Scattering
QC-MVP-0011	Method Validation Protocol of SOP-0998: Determination of Particle Size Distribution and Polydispersity by Dynamic Light Scattering

10. Attachments

Attachment 1: QC-MVR-0011 Data Portfolio (Veeva)

Attachment 2: QC-MVR-0011 Verified Excel Data (Veeva)

Attachment 3: QC-MVR-0011 Excel Data (Veeva)

11. Revision History

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

Document Approvals

Approved Date: 21 Sep 2020

Task: Approval Task Verdict: Approve	[redacted]	

Task: QA Approval Task Verdict: Approve	[redacted]	