Number: QC-MVR-0011 Version: 1.0 Approved Date: 21 Sep 2020

Method Validation Report of SOP-0998

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Method Validation Report

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

mRNA-1273 LNP, mRNA-1273 DP,

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), 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, 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		
	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 Sultability	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	The average normalized intensity from the blank PBS sample result was	Pass



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Parameter	Acceptance Criteria	Results	Pass / Fai			
	For each size standard, the %RSD of the triplicate diameter (nm) readings must be	Particle Size (nm) %RSD:				
Accuracy	For each size standard, the difference between CoA and the average measured particle size value must be	Std Difference against CoA:	Pass			
	PDI for all measurements	PDI for all measurements:				
	The particle size %RSD of					
	of an Redu	mRNA-1273 DP Lot 6006820001				
	The particle size %RSD of the 6 replicate values for each test article must be	mRNA-1273 DP Lot 6006920001				
3	80 8603	Analyst 1 PDI values:	Pass			
	Report the individual and mean of the polydispersity	Sample Prep Pdl Average Pdl				
Precision Repeatability)	index (PdI) of the 6 replicate values for each test article	1 2				
		mRNA-1273 3 LNP Lot 4 5006820001 5				
		6				

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Parameter	Acceptance Criteria		Result	S	ನ	Pas / Fa
		Sample	Prep	Pdl	Average Pdl	
			1 2			
		mRNA-1273	3			
		DP Lot 6006820001	4			
			5 0			
			5			
Precision			Prep	Pdl	Average	
Repeatability)		000 5010 000	1		Pdl	
	, e	"Holls You	2			
	SLUG S	mRNA-1273 DP Lot 6006920001	3			
	Letine		5			
	Mariation		6			
	and edu.				Average	
	Solger,	Sample	Prep	Pdl	Pdl	
	"O SOLD THE		2			
d	30,835	Lot				
ve)	501	AMPDP-200062	5			
			6			
N. CO.	For Analyst 2, the particle size %RSD of the 6 replicate values for each test article must be		·			
	replicate values for each					
Precision	test article must be For Analyst 1 and 2, the					Pas
Intermediate)	particle size %RSD of					
	sample replicates (n=12) for each test article must					



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arameter	Acceptance Criteria	Results	Pas / Fa
		Analyst 2 Particle Size (nm) %RSD:	2
		Sample %RSI	ם כ
		mRNA-1273 LNP Lot 5006820001	
		mRNA-1273 DP Lot 6006820001	
		mRNA-1273 DP Lot 6006920001	
		Lot AMPDP-200062	
	The % Difference of mean	Analyst 1 and 2 Particle Size (nm) %RS	D:
	particle size between Analyst 1 and 2	Sample %RSI	
	For Analyst 1 and 2, report the individual and mean of	mRNA-1273 LNP Lot 5006820001	
	the polydispersity index	mRNA-1273 DP Lot 6006820001	
repl	icate (n=12) values for each test article.	mRNA-1273 DP Lot 6006920001	
	SUA BOTHS	Lot AMPDP-200062	
	eplicate (n=12) values for each test article.	% Difference of mean particle size (nm between Analyst 1 and 2:)
	2,03,60	Sample %Dif	f
Š	C660	mRNA-1273 LNP Lot 5006820001	
		mRNA-1273 DP Lot 6006820001	
		mRNA-1273 DP Lot 6006920001	
		Lot AMPDP-200062	

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Parameter	Acceptance Criteria		Results	3		P:
		Analyst 2 and	l combir	ned PDI	values:	
		Sample	Prep	Pdl	Average Pdl	
		mRNA-1273 LNP Lot 5006820001 A1 and A	1 2 3 4 5 6 2 (n=12)			
Precision ntermediate)		Sample	Pren	Pdl	Average	
	en o		1	1 61	Pdl	
	Cill's		2			
	all allo	mRNA-1273 DP	3			
	any adula	Lot 6006820001	4			
	Ka. Lo		5			
	1000 yes	A1 and A	6 2 (n=12)			
	10 301/11	AT and A	2 (11–12)			
ુંડ	ad to support any marketing ad to support any marketing ad to support any marketing any marketing and a support a support and a support a	Sample	Prep	Pdl	Average Pdl	
7.00			1			
			2			
Co		mRNA-1273 DP	3			
		Lot 6006920001	4			
			5 6			
		A1 and A				
			/			

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mRNA-1273 LNP, mRNA-1273 DP,

Parameter	Acceptance Criteria	Results					
			M 1000000000000000000000000000000000000		:003		
		Sample	Prep	Pdl	Average Pdl		
			1				
			3				
		AMPDP-200062	4				
			6				
		A1 and A	2 (n=12)				
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	A1 and A	ge:		•	Pass	
Robustness	accuracy. Intermediate precision criteria are met.	Intermediate p	recision o	criteria	were met.	Pass	

6. Validation Results

6.1. Specificity and Accuracy

Experimental Design:

Triplicate preparations each of the particle size standards 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 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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	od Validation Report of SOP-0998: Determination of Particle Size Distribution and dispersity by Dynamic Light Scattering
mRN	A-1273 LNP, mRNA-1273 DP,
	The average particle size for each triplicate reading for the standards was calculated. For a size standard to be included in the accuracy and range determination, the %RSD must be and the mean diameter must be 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
	Accuracy Acceptance Criteria:
	For each size standard, the %RSD of the triplicate diameter (nm) readings must be
	For each size standard, the difference between CoA and the average measured particle size value must be Specificity Results
	The average normalized intensity from the blank PBS sample result was Results are presented in Table 1. The results demonstrate that there is no interference due to the blank PBS sample.
	Table 1. Specificity Results Average Normalized Intensity (kCnts/s) Accuracy Results
	Accuracy Results
ant col	For each size standard, the %RSD of the triplicate diameter (nm) reading was standards assessed
(o.	For each size standard, the average measured particle size value was within 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

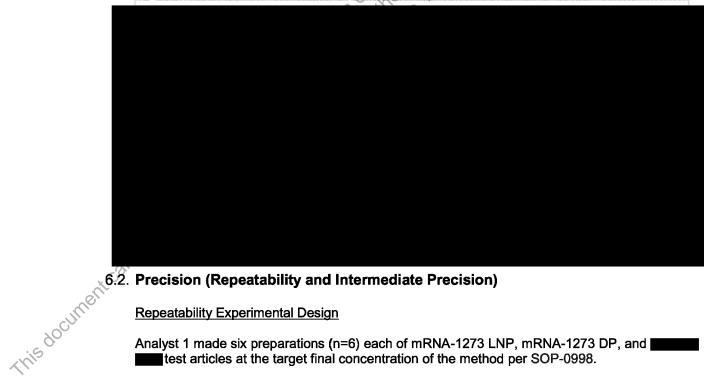
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mRNA-1273 LNP, mRNA-1273 DP,

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
		1					
		2					
		3					
		1					
		2					
		3					
		1					
		2					
		3					

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



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

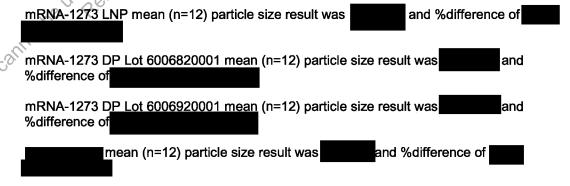
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mRNA-1273 LNP,	mRNA-1273 DP,		.*.
Intermediate	Precision Experimental Design	1	Valiat
using differen		epeatability precision assay on a se I sample dispersants than Analyst 1. ediate precision assay.	. The same lots
Precision data	a from Analyst 1 and Analyst 2	ediate precision assay. ! were combined for n=12.	2
The particle s	ize and polydispersity index (F	Pdl) for each replicate was reported	for each analys
The %RSD o	the 6 replicate values for each	h test article was calculated for each	n analyst.
The %RSD o		ch test article was calculated for an	alyst 1 and 2.
Acceptance (30.	asin 200	
	and all	the 6 replicate values for each test	
For Analyst 1 mean particle	and 2, the particle size %RSD size for each test article must	of <u>sample replicates</u> (n=12) and % be	difference of
For Analyst 1	and 2, report the individual an	d mean of the sample polydispersity	y 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 -



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

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

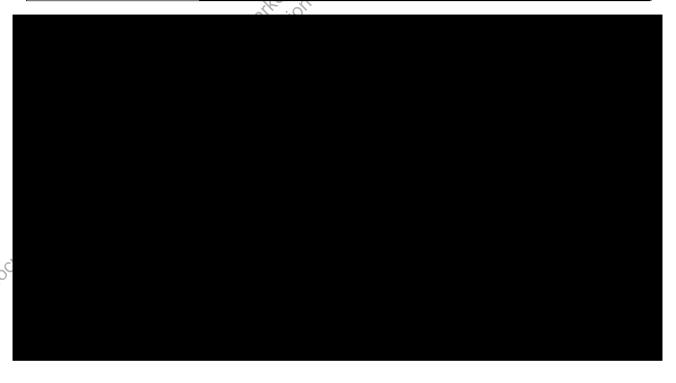
Table 4. Precision Results for mRNA-1273 DP Lot 6006820001

mRNA-1273 DP Lot 6006820001	Prep	Particle Size	Pdl	Average Particle Size	Average Pdl	%RSD Particle Size	% Difference
Analyst 1	2 3 4			125 Marie de de la constante d		ve jest kan	
Mott	5 6						
crit con	1						
0	2						
Analyst 2	3						
Analyst Z	4						
	5						
	6						
Analyst 1&	2						

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

mRNA-1273 DP Lot 6006920001	Prep	Particle Size	Pdl	Average Particle Size	Average PdI	%RSD Particle Size	% Difference
	1						
	2						
Amelyot d	3						
Analyst 1	4						
	5						
	6						
	1						
	2						
Amelyot 2	3						
Analyst 2	4						
	5						
	6						
Analyst 1&	2						



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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 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.

7. Discrepancies

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, |

The validated particle size range 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.

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mRNA-1273 LNP, mRNA-1273 DP,

9. Referenced Documents

Document #	
ICH Q2(R1)	International Council for Harmonization, Validation of Analytical Procedures
FRM-0728	SOP-0998 Assay Performance Worksheet
TR-9540	- R+U - SOP-0998 v0.2 and QC-MVP-0011 v.1.0
TR-9541	- 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 2: QC-MVR-0011 Data Portfolio (Veeva)

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

Revision Line

11. Revision History

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