



Australian Government
 Department of Health and Aged Care
 Therapeutic Goods Administration

Laboratories Branch

Type: Biotherapeutics\BPC\Forms	Number: Bio-BPC-Form-10 / Version: 2
Owner: s22	Approver: s22
Active: 28/07/2023	Review: 20/07/2022
Title: HPLC – General Worksheet	

HPLC General Worksheet

Test Details	
Test Name	Identification and Quantitation of PEG 2000 DMG, Cholesterol, SM-102 and DSPC in Moderna Vaccine
Method Reference	Bio-BPC-Method-38 Version: 2
Method modifications (if any)	N/A
Modifications approved by	N/A
Data location in TRIM	D23-4039146
Name of analyst	s22
Test Date	7/11/2023

Buffers and Solutions	
Solutions	Batch number
Mobile Phase A	CP06Nov23-1, Exp: 06Dec23
Mobile Phase B	CP06Nov23-2, Exp: 06Dec23
Ethanol (ACS Grade)	Supelco, I1262583305
Comments:	

Pipettes used and expiry dates	
33188	25/12/2023
33438	21/02/2024

Reference Materials	
Name and Code	Batch number
Moderna Lipid Stock A	18May23, D23-5410602 (Assay Control), Exp: 18Nov23
Moderna Lipid Stock B	18May23, D23-5410602 (Calibration Curve), Exp: 18Nov23
Comments: Assay Control: 1:3 Diluted 250uL of assay control in 750uL in ethanol. Calibration Curve prepared as per SOP.	

NOTES
Comments: Samples were removed from -20°C freezer at 12:40pm and sampled at 1:30pm the same day.

System Suitability Criteria and Results			
Parameters	Limits	Results	Comments
No significant interference peaks should be observed between 4 and 22 minutes in the last diluent as blank injection	No interfering peaks	No interfering peaks	Pass
The RSD of peak areas of the first three SST injections	≤5.0%	All four lipids <1%	Pass
The RSD of retention times of the first three SST injections	≤5.0%	All four lipids <1%	Pass
The RSD of peak areas for the SST injections during the run	≤5.0%	All four lipids <1%	Pass
The RSD of retention times for the SST injections during the run	≤5.0%	All four lipids <2%	Pass
The r ² of the standard curve	≥0.995 for all lipids	≥0.995 for all four lipids	Pass
Percentage recovery for the lipids in 1:3 assay control	85-115%	All four lipids 99-101%	Pass

Sample 1					
Sample Name	SPIKEVAX XBB.1.5 (andusomeran) COVID-19 VACCINE 0.1 mg/mL suspension for injection pre-filled syringe				
LIMS No:	2311003783	Batch No:	3034111		
Sample Dilutions and Calculations					
Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.
N/A	250 µL	750 µL	N/A	4	20 µL
Comments:					
Two separate syringes were used and prepared as per SOP.					
Sample Acceptance Criteria					
Parameters	Limits	Results	Comments		
% Difference for sample preparations is NMT 5%	≤5.0%	PEG: 1.1 Cholesterol: 1.0 SM102: 0.7 DSPC: 3.0	Pass		
Peak shape	Peak shapes sample match SST	Peak shapes match SST for all four lipids	Pass		
Test Results					
Parameters	Limits	Results	Comments		
% RT difference between sample preparations and SST	95-105%	All four lipids 98-101%	Pass		
PEG2000-DMG	s47		Pass		
Cholesterol			Pass		
SM102			Pass		
DSPC			Pass		

Sample 3					
Sample Name					
LIMS No:		Batch No:			
Sample Dilutions and Calculations					
Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.
Comments:					
Sample Acceptance Criteria					
Parameters	Limits	Results	Comments		
Test Results					
Parameters	Limits	Results	Comments		

Sample 4					
Sample Name					
LIMS No:				Batch No:	
Sample Dilutions and Calculations					
Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.
Comments:					
Sample Acceptance Criteria					
Parameters	Limits	Results	Comments		
Test Results					
Parameters	Limits	Results	Comments		

Sample 5					
Sample Name					
LIMS No:				Batch No:	
Sample Dilutions and Calculations					
Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.
Comments:					
Sample Acceptance Criteria					
Parameters	Limits	Results	Comments		
Test Results					
Parameters	Limits	Results	Comments		

Sample 6					
Sample Name					
LIMS No:		Batch No:			
Sample Dilutions and Calculations					
Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.
Comments:					
Sample Acceptance Criteria					
Parameters	Limits	Results	Comments		
Test Results					
Parameters	Limits	Results	Comments		



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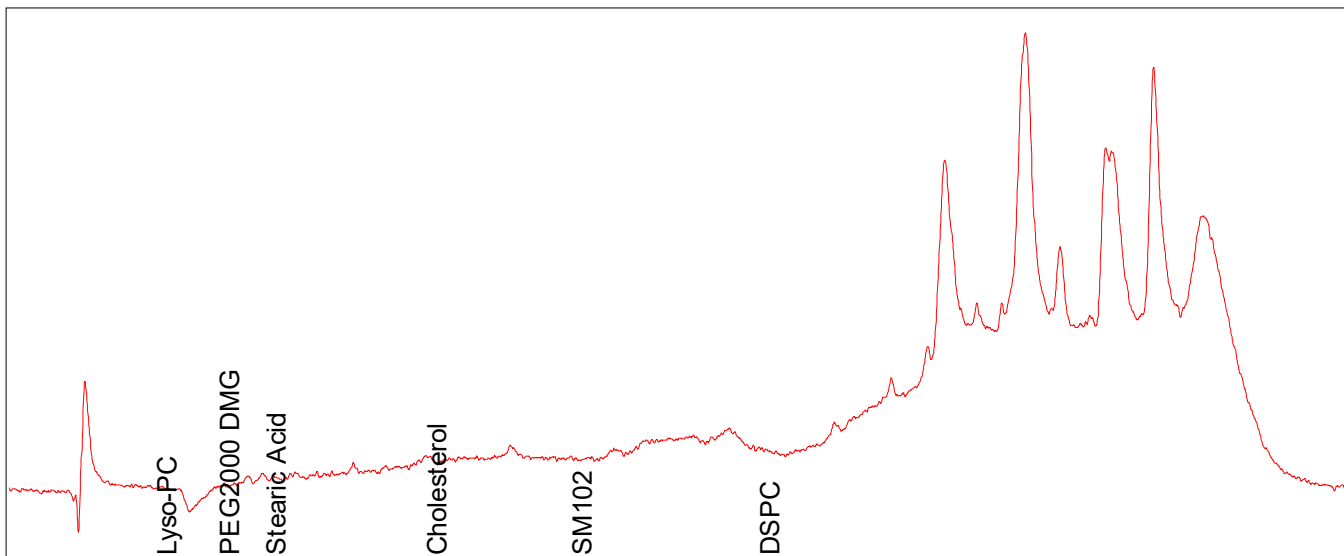
STD-Level 1 , STD-Level 4 , Blank EtOH, 2311003783 -
P1, s22 - P1, STD-Level 6, 2311003783 - P2, SST,
STD-Level 5 . s22 - P2. Assav Control 1:3 .

Sample Set Name: Moderna 11Nov23 s22

Sample Set Acquired By: s22

System Suitability

Blank Ethanol Injection

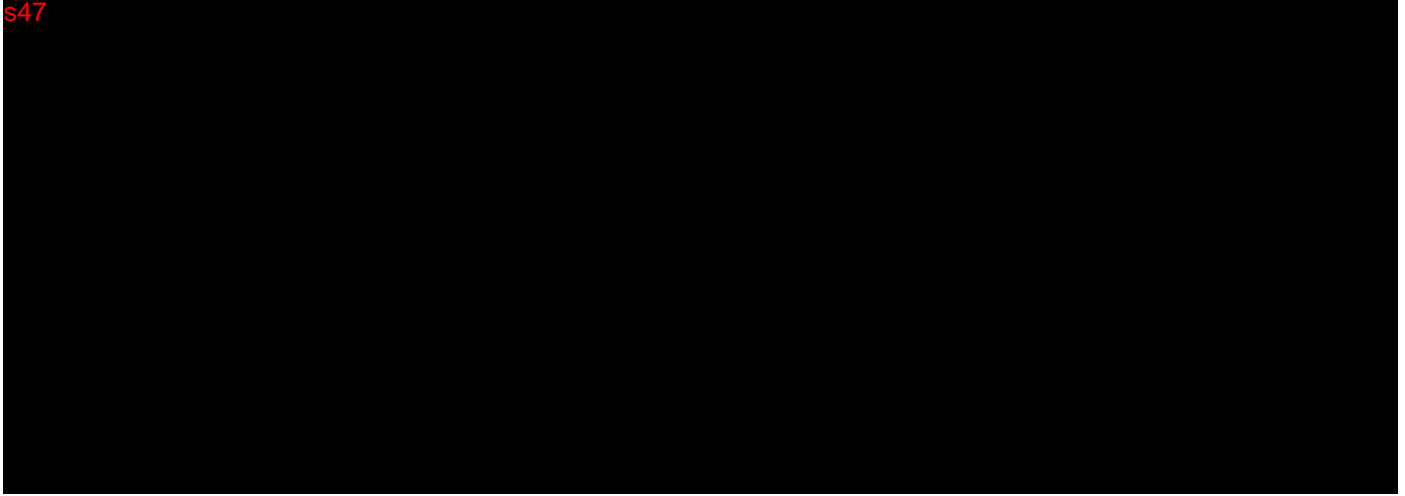


— Label B1; SampleName: Blank EtOH; Vial: 1:A,1; Injection: 1

Component Results
% Area Summarized by Name

SampleName	Lyso-PC	PEG2000 DMG	Stearic Acid	Cholesterol	SM102	DSPC
1 BlankEtOH						

First three SST injections



- Label Y1; SampleName: SST; Vial: 1:A,4; Injection: 1
- Label Y1; SampleName: SST; Vial: 1:A,4; Injection: 2
- Label Y1; SampleName: SST; Vial: 1:A,4; Injection: 3

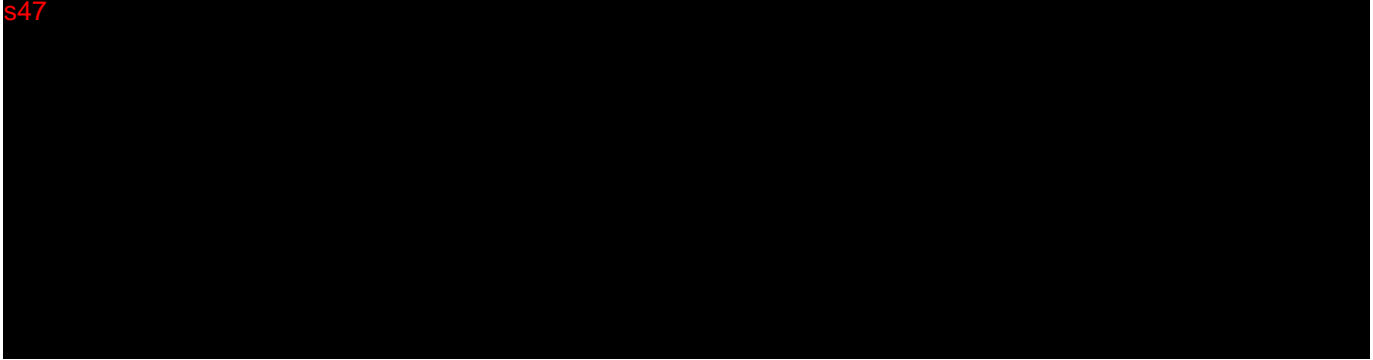
Component Results
Area Summarized by Name

	SampleName	Label	PEG2000 DMG ($\mu\text{V}\cdot\text{sec}$)	Cholesterol ($\mu\text{V}\cdot\text{sec}$)	SM102 ($\mu\text{V}\cdot\text{sec}$)	DSPC ($\mu\text{V}\cdot\text{sec}$)
1	SST	Y1	s47			
2	SST	Y1				
3	SST	Y1				
Mean						
% RSD			0.2	0.3	0.0	0.4

Component Results
Retention Time Summarized by Name

	SampleName	Label	PEG2000 DMG (min)	Cholesterol (min)	SM102 (min)	DSPC (min)
1	SST	Y1	5.020	9.657	13.023	17.181
2	SST	Y1	5.036	9.658	12.985	17.179
3	SST	Y1	5.038	9.656	12.981	17.185
Mean			5.031	9.657	12.996	17.182
% RSD			0.197	0.013	0.178	0.018

First three and bracketing SST injections



- Label Y1; SampleName: SST; Vial: 1:A,4; Injection: 1
- Label Y1; SampleName: SST; Vial: 1:A,4; Injection: 2
- Label Y1; SampleName: SST; Vial: 1:A,4; Injection: 3
- Label Y2; SampleName: SST; Vial: 1:A,4; Injection: 1
- Label Y2; SampleName: SST; Vial: 1:A,4; Injection: 2
- Label Y3; SampleName: SST; Vial: 1:A,4; Injection: 1
- Label Y3; SampleName: SST; Vial: 1:A,4; Injection: 2

Component Results
Area Summarized by Name

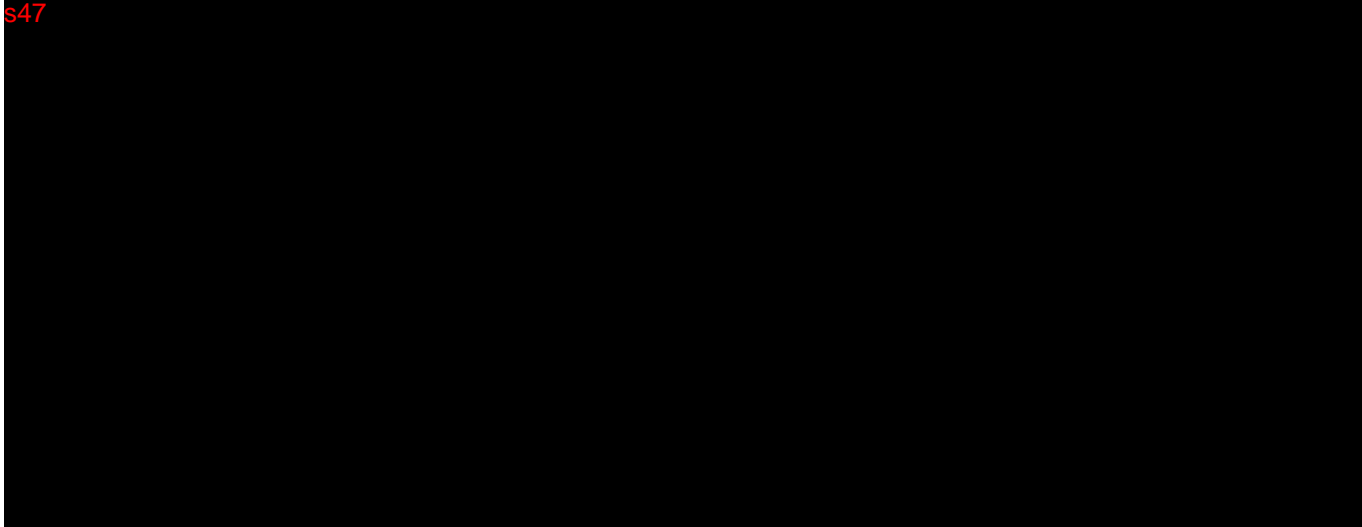
	SampleName	Label	PEG2000 DMG ($\mu\text{V}\cdot\text{sec}$)	Cholesterol ($\mu\text{V}\cdot\text{sec}$)	SM102 ($\mu\text{V}\cdot\text{sec}$)	DSPC ($\mu\text{V}\cdot\text{sec}$)
1	SST	Y1	S47			
2	SST	Y2				
3	SST	Y3				
4	SST	Y2				
5	SST	Y1				
6	SST	Y1				
7	SST	Y3				
Mean						
% RSD			0.7	0.6	0.7	0.6

Component Results
Retention Time Summarized by Name

	SampleName	Label	PEG2000 DMG (min)	Cholesterol (min)	SM102 (min)	DSPC (min)
1	SST	Y1	5.020	9.657	13.023	17.181
2	SST	Y2	5.027	9.658	13.232	17.224
3	SST	Y3	5.028	9.666	13.270	17.228
4	SST	Y2	5.035	9.674	13.251	17.238
5	SST	Y1	5.036	9.658	12.985	17.179
6	SST	Y1	5.038	9.656	12.981	17.185
7	SST	Y3	5.038	9.653	13.267	17.197
Mean			5.031	9.660	13.144	17.205
% RSD			0.1	0.1	1.1	0.1

Assay Control

s47



— Label C; SampleName: Assay Control 1:3 ; Vial: 1:A,8; Injection: 1
 — Label C; SampleName: Assay Control 1:3 ; Vial: 1:A,8; Injection: 2

Component Results

Percent LipidRec_Conc Summarized by Name

	SampleName	Label	PEG2000 DMG	Cholesterol	SM102	DSPC
1	Assay Control 1:3	C	99.51	100.27	98.78	100.01
2	Assay Control 1:3	C	99.66	100.45	99.26	100.34
Mean			99.6	100.4	99.0	100.2
% RSD			0.1	0.1	0.3	0.2

Calibration Curve

System Suitability Summary Results
Name: Cholesterol

	Name	Sample Name	R^2	Concentration
1	Cholesterol	STD-Level 1	0.999910	s47
2	Cholesterol	STD-Level 5	0.999910	

System Suitability Summary Results
Name: DSPC

	Name	Sample Name	R^2	Concentration
1	DSPC	STD-Level 1	0.999920	s47
2	DSPC	STD-Level 5	0.999920	

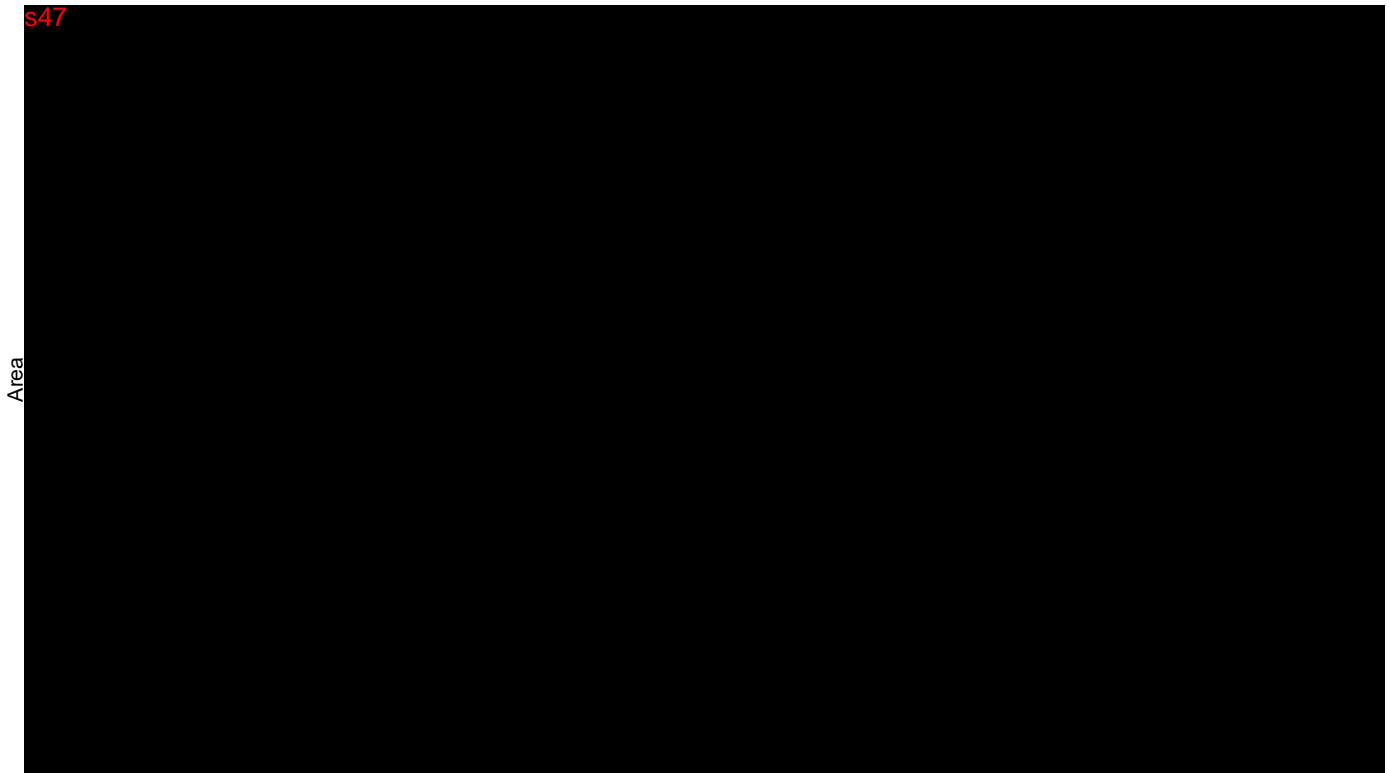
System Suitability Summary Results
Name: PEG2000 DMG

	Name	Sample Name	R^2	Concentration
1	PEG2000 DMG	STD-Level 1	0.999960	s47
2	PEG2000 DMG	STD-Level 5	0.999960	

System Suitability Summary Results
Name: SM102

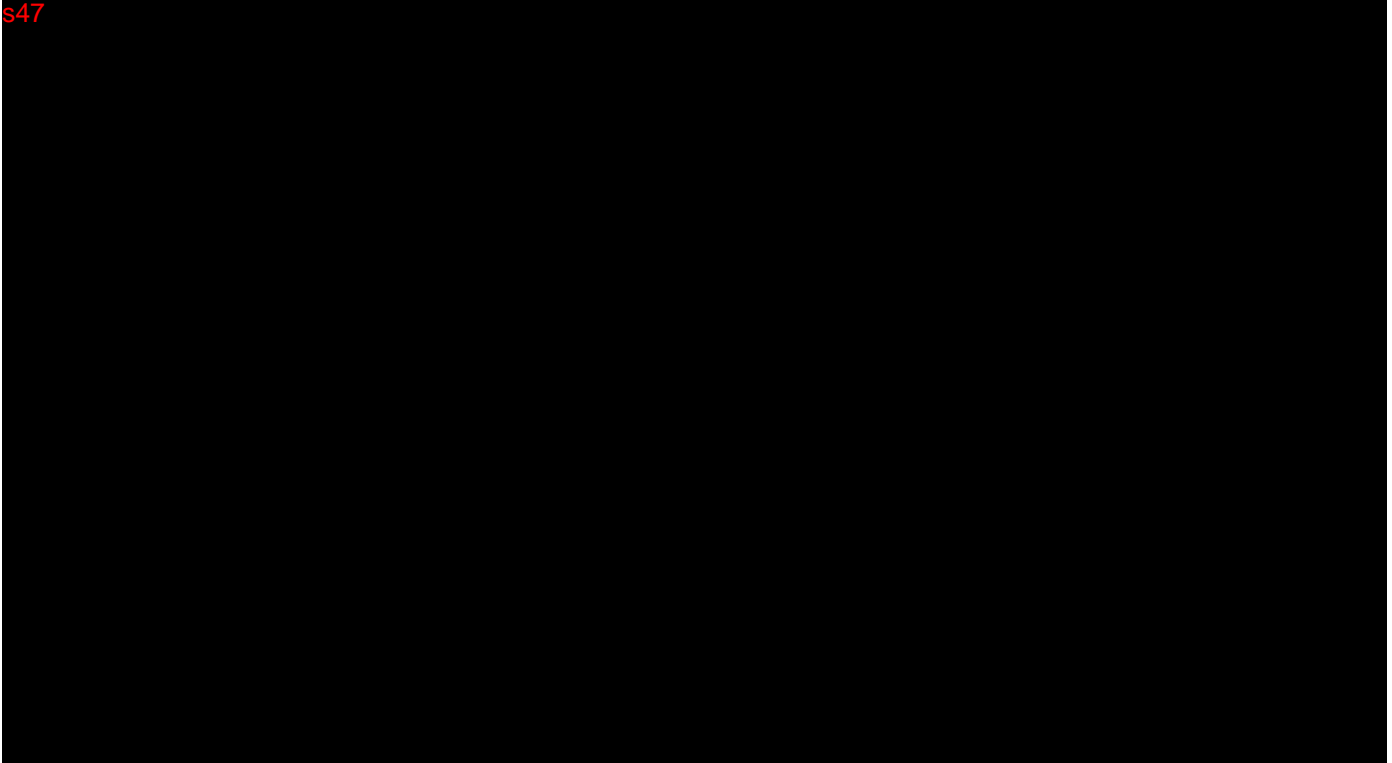
	Name	Sample Name	R^2	Concentration
1	SM102	STD-Level 1	0.999941	s47
2	SM102	STD-Level 5	0.999941	

Calibration Plot group for Lyso-PC contains no data.



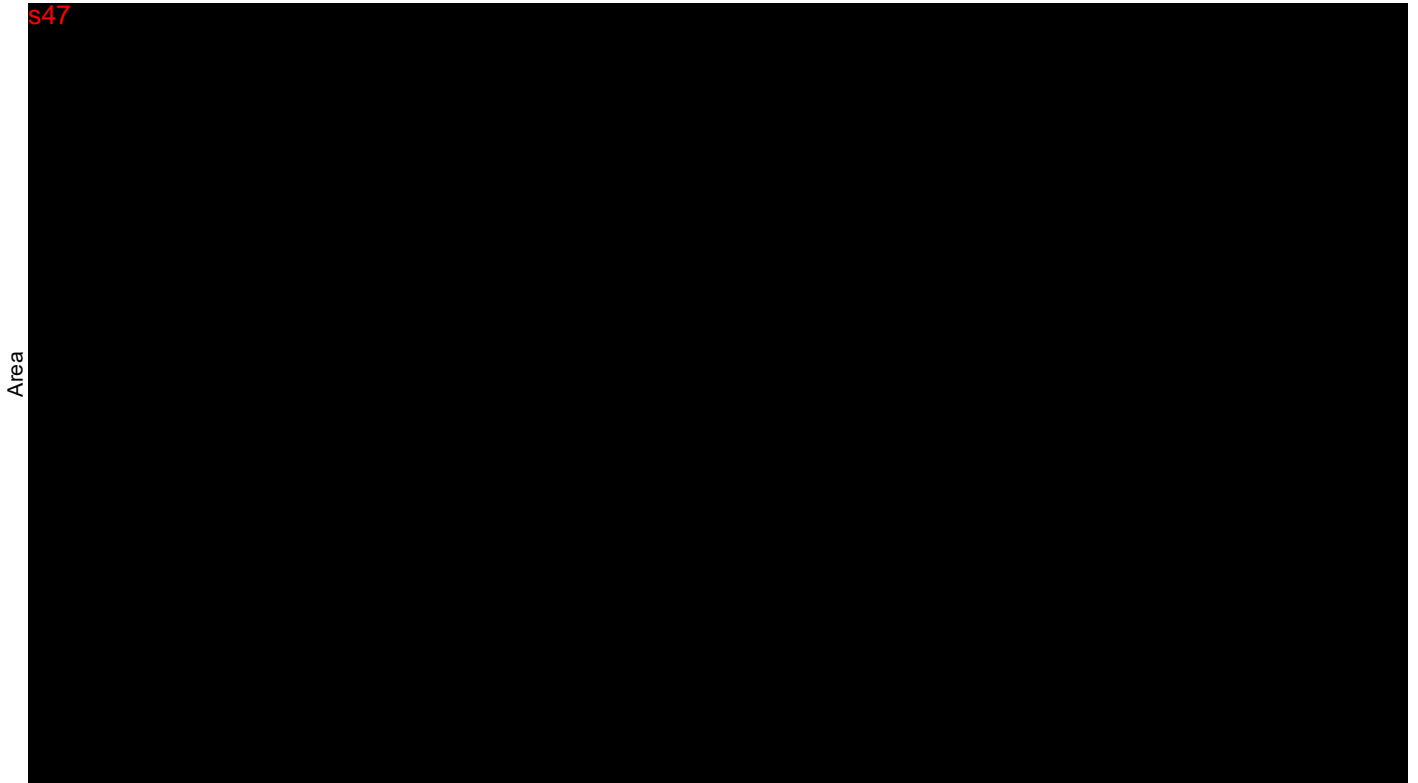
Name: PEG2000 DMG; Processing Method: Moderna_CP; Fit Type: Quadratic (2nd Order); Cal
Curve Id: 3248; A: 4.873271e+03; B: 1.205363e+06; C: -5.407892e+04; D: 0.000000e+00; R^2:
0.999960

Calibration Plot group for Stearic Acid contains no data.



———— Name: Cholesterol; Processing Method: Moderna_CP; Fit Type: Quadratic (2nd Order); Cal Curve
Id: 3250; A: -3.702273e+04; B: 9.736359e+05; C: -1.158587e+04; D: 0.000000e+00; R^2:
0.999910

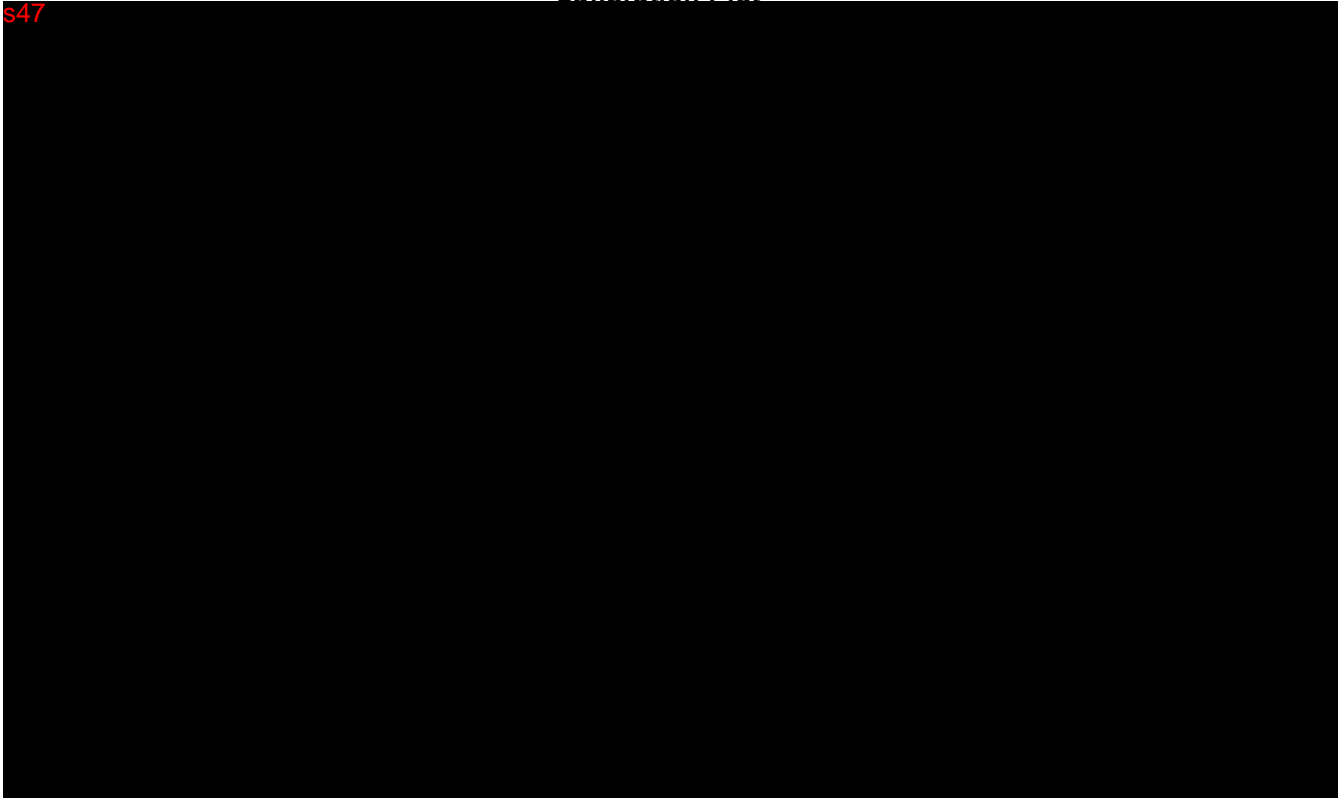
Calibration Plot



Name: SM102; Processing Method: Moderna_CP; Fit Type: Quadratic (2nd Order); Cal Curve Id: 3251; A: 1.747904e+05; B: 1.005860e+06; C: -9.046512e+03; D: 0.000000e+00; R^2: 0.999941

Calibration Plot

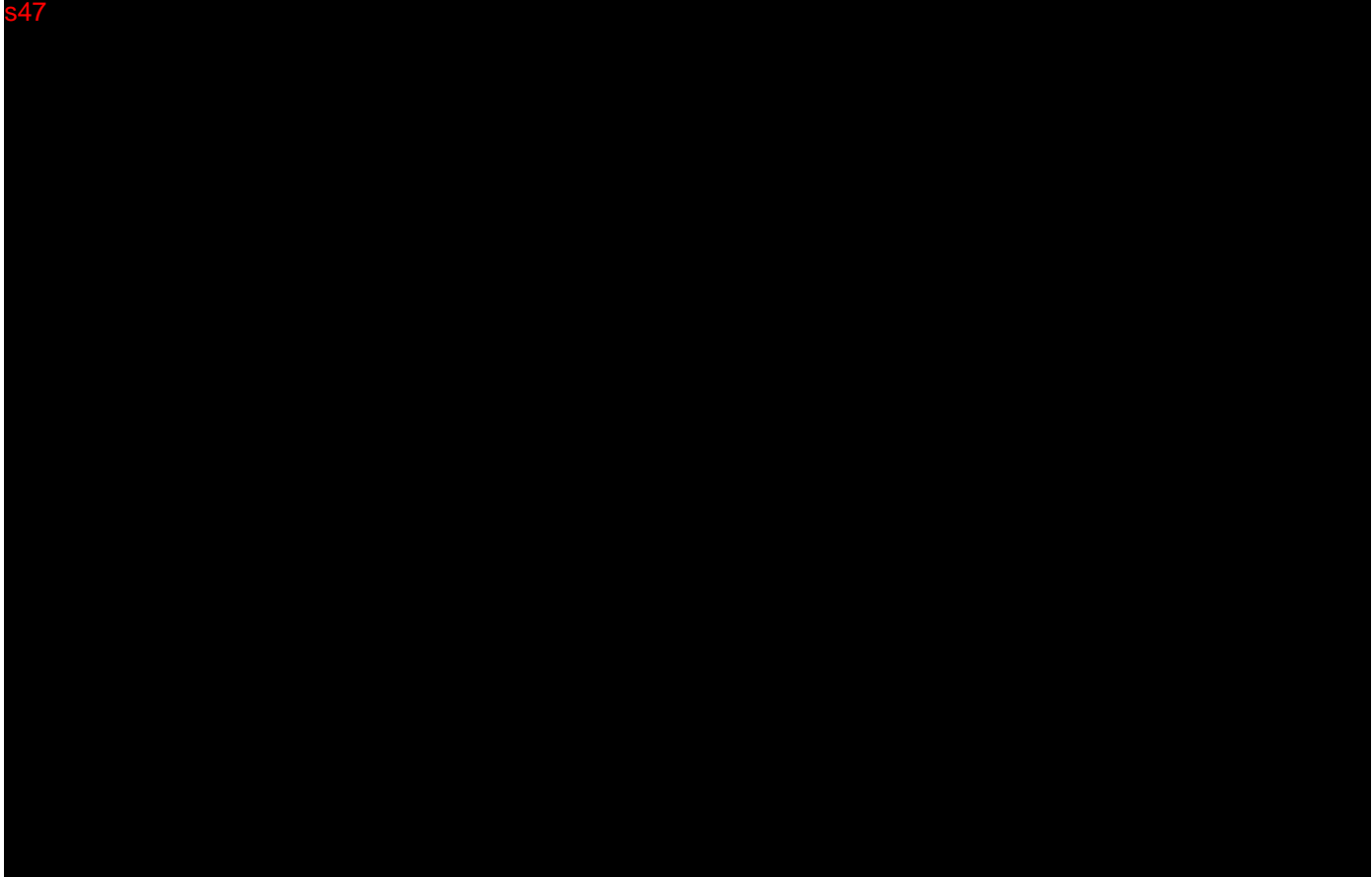
s47



Name: DSPC; Processing Method: Moderna_CP; Fit Type: Quadratic (2nd Order); Cal Curve Id: 3252; A: 6.332263e+03; B: 1.129864e+06; C: -3.760037e+04; D: 0.000000e+00; R^2: 0.999920

Sample Results

Sample 1



- SampleName: 2311003783 - P1; Vial: 1:B,1; Injection: 1; Label U101
- SampleName: 2311003783 - P1; Vial: 1:B,1; Injection: 2; Label U101
- SampleName: 2311003783 - P2; Vial: 1:B,2; Injection: 1; Label U102
- SampleName: 2311003783 - P2; Vial: 1:B,2; Injection: 2; Label U102

Concentration Summarized by Name

	Label	SampleName	Inj	PEG2000 DMG	Cholesterol	SM102	DSPC
1	U101	2311003783 - P1	1	s47			
2	U101	2311003783 - P1	2				
3	U102	2311003783 - P2	1				
4	U102	2311003783 - P2	2				
Mean							
% RSD				1.3	0.7	0.5	1.7

RT_Ratio Summarized by Name

	Label	SampleName	Inj	PEG2000 DMG	Cholesterol	SM102	DSPC
1	U101	2311003783 - P1	1	98.2	100.5	100.6	100.3
2	U101	2311003783 - P1	2	99.1	100.4	100.8	100.3
3	U102	2311003783 - P2	1	97.6	100.4	100.8	100.2
4	U102	2311003783 - P2	2	98.4	100.4	100.9	100.3
Mean				98.3	100.4	100.8	100.3
% RSD				0.6	0.0	0.1	0.0

SampleName STD-Level 1 , STD-Level 4 , Blank EtOH, 2311003783 - P1, s22 - P1, STD-Level 6, 2311003783 - P2, SST, STD-Level 5 , s22 - P2, Assay Control 1:3 , STD-Level 3 , STD-Level 2

U1_Percent_differ Summarized by Name

	Label	SampleName	Inj	PEG2000 DMG	Cholesterol	SM102	DSPC
1	U101	2311003783 - P1	1	s47			
2	U101	2311003783 - P1	2				
3	U102	2311003783 - P2	1				
4	U102	2311003783 - P2	2				
Mean							
% RSD				0.0	0.0	0.0	0.0



Australian Government

Department of Health and Ageing
Therapeutic Goods Administration

Sample Set Summary Report

Sample Set: Moderna 11Nov23 s22

Sample Set Information

Project Name: Biochemistry\2023\SpikeVax Lipids

Sample Set Name: Moderna 11Nov23 s22

SampleName: STD-Level 1 , STD-Level 4 , Blank EtOH,
2311003783 - P1, s22 - P1, STD-Level 6,
2311003783 - P2, SST, STD-Level 5 , s22 - P2,
Assay Control 1:3 , STD-Level 3 , STD-Level 2

Sample Set Acquired By: s22

Start Date: 7/11/2023 2:11:51 PM AEDT

Finish Date: 8/11/2023 10:46:44 AM AEDT

Acq Method Set: Moderna Vaccine

Run Time: 30.00 Minutes

Instrument Method Name: Moderna Vaccine

Sample Set Altered: Yes

Sample Set Method: BT_SpikeVax Lipid_Sample Set

System Information

System Name: System 20 esatin only @jnpl7c2

Analytical_Column_1: unlisted column

Empower Node: Ucdpjnpl7c2

Analytical_Column_2:

Processing Information

Processing Method: Moderna_CP

Result Set Name: Moderna 11Nov23 s22

Processed By: s22/Biochem

Result Set Date: 8/11/2023 11:47:40 AM AEDT

Processing Method Id: 2971

Result Set Id: 3245

Date Processed: 8/11/2023 11:47:42 AM AEDT,
8/11/2023 11:47:44 AM AEDT, 8/11/2023 11:47:45

Channel Description: Channel 1

Processing Node: Uclp4v71dk3

Reporting Information

Report Method Name: Sample Set Summary Report

Print Date: 8/11/2023

Reported by: s22

Time: 11:56:27 AM Australia/Canberra

Injection Sequence Summary

	SampleName	Sample Type	Vial	Inj #	Run Time (Minutes)	Injection Volume (ul)	Sample Weight	Dilution	Level	Label
1	Blank EtOH	Control	1:A,1	1	30.00	10.00	1.00000	1.00000		B1
2	SST	Control	1:A,4	1	30.00	10.00	1.00000	2.50000		Y1
3	SST	Control	1:A,4	2	30.00	10.00	1.00000	2.50000		Y1
4	SST	Control	1:A,4	3	30.00	10.00	1.00000	2.50000		Y1
5	STD-Level 1	Standard	1:A,2	1	30.00	10.00	1.00000	10.00000	Level 1	S1
6	STD-Level 1	Standard	1:A,2	2	30.00	10.00	1.00000	10.00000	Level 1	S1
7	STD-Level 2	Standard	1:A,3	1	30.00	10.00	1.00000	5.00000	Level 2	S2
8	STD-Level 2	Standard	1:A,3	2	30.00	10.00	1.00000	5.00000	Level 2	S2
9	STD-Level 3	Standard	1:A,4	1	30.00	10.00	1.00000	2.50000	Level 3	S3
10	STD-Level 3	Standard	1:A,4	2	30.00	10.00	1.00000	2.50000	Level 3	S3
11	STD-Level 4	Standard	1:A,5	1	30.00	10.00	1.00000	1.66670	Level 4	S4
12	STD-Level 4	Standard	1:A,5	2	30.00	10.00	1.00000	1.66670	Level 4	S4
13	STD-Level 5	Standard	1:A,6	1	30.00	10.00	1.00000	1.25000	Level 5	S5
14	STD-Level 5	Standard	1:A,6	2	30.00	10.00	1.00000	1.25000	Level 5	S5
15	STD-Level 6	Standard	1:A,7	1	30.00	10.00	1.00000	1.00000	Level 6	S6
16	STD-Level 6	Standard	1:A,7	2	30.00	10.00	1.00000	1.00000	Level 6	S6
17	Assay Control 1:3	Control	1:A,8	1	30.00	20.00	1.00000	4.00000		C
18	Assay Control 1:3	Control	1:A,8	2	30.00	20.00	1.00000	4.00000		C
19	2311003783 - P1	Unknown	1:B,1	1	30.00	20.00	1.00000	4.00000		U101
20	2311003783 - P1	Unknown	1:B,1	2	30.00	20.00	1.00000	4.00000		U101
21	2311003783 - P2	Unknown	1:B,2	1	30.00	20.00	1.00000	4.00000		U102
22	2311003783 - P2	Unknown	1:B,2	2	30.00	20.00	1.00000	4.00000		U102
23	SST	Control	1:A,4	1	30.00	10.00	1.00000	2.50000		Y2
24	SST	Control	1:A,4	2	30.00	10.00	1.00000	2.50000		Y2
25	s22									
26										
27										
28										
29	SST	Control	1:A,4	1	30.00	10.00	1.00000	2.50000		Y3
30	SST	Control	1:A,4	2	30.00	10.00	1.00000	2.50000		Y3



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Laboratories Branch

Owner: s22	Number: Chem-Form-6
Author: s22	Version: 1
Active: 27/06/2021	Review: <QPulse_DocReviewDate>
Title: Analysts notes - Worksheet	

ANALYSTS' NOTES

Sample Name Spikevax (Moderna)

TGA Sample No. 2109003344

Lot number: 000002A

SUBSTANCES ASSAYED: PEG 2000 DMG, Cholesterol, SM102 and DSPC.

Method reference: Chem-Method-45

INSTRUMENT OR SYSTEM No. 20 with CAD detector

SYSTEM SUITABILITY REQUIREMENTS MET (Y/N) ? YES **ASSAY REQUIREMENTS MET (Y/N)? YES**

TGA Sample number	Preparation	Lipid content (mg/mL)			
		PEG 2000 DMG	Cholesterol	SM 102	DSPC
	1	s47			
	2				
Average	-				

ATTACHMENTS:

System suitability report, Calibration plots, Result summary report, Example chromatograms, Run and method summary and mobile phase preparation details.

Identification RT requirements met ? (RT ± 5% compared to SST) (Y/N) yes

RESULT

PASS ✓

FAIL ☒

Signature of Analyst

Signed electronically s22

Date 19 Sep 2021

Checked by Official Analyst...Signed electronically by s22

...Date...19/09/21...



Australian Government
Department of Health
 Therapeutic Goods Administration

Laboratories Branch

Owner: s22	Number: Chem-Form-6
Author: s22	Version: 1
Active: 27/06/2021	Review: <QPulse_DocReviewDate>
Title: Analysts notes - Worksheet	

ANALYSTS' NOTES

Sample Name Spikevax (Moderna)

TGA Sample No. 2109003344

WORKING STANDARD CURVE PREPARATION DETAILS

Date Stock solution prepared: 26 Aug 2021 **Prepared by:** s22 **Within expiry Y/N**—yes

Attached **or Refer to (TRIM Record number/Sample number)** D21-3106881

Lipid concentrations in the mixed lipid stock solution

Lipid Standard	weight in mg	stock soln (mg/mL)	Purity
PEG2000DMG (6548)	s47		94
Cholesterol (6546)	s47		99
SM102 (6547)	s47		99.7
DSPC (6542)	s47		99

Calibration curve standards solution preparation

Prepared by: s22 .**Date prepared** 18 Sep 2021 POVA LIMS: 33271

Linearity level	STD Stock sol (µL)	EtOH (µL)	Concentration of total lipid (mg/mL)
Level 6 (250%)	STD stock Solution		s47
Level 5 (200%)	800	200	s47
Level 4 (150%)	600	400	s47
Level 3 (100%)	400	600	s47
Level 2 (50%)	200	800	s47
Level 1 (25%)	100	900	s47

Signature of Analyst

Signed electronically s22

Date 19 Sep 2021

Checked by Official Analyst... Signed electronically by s22

.....Date... 19/09/21



Australian Government

Laboratories Branch

Department of Health

Therapeutic Goods Administration

Owner: s22	Number: Chem-Form-6
Author: s22	Version: 1
Active: 27/06/2021	Review: <QPulse_DocReviewDate>
Title: Analysts notes - Worksheet	

ANALYSTS' NOTES

Sample Name Spikevax (Moderna)

TGA Sample No. 2109003344

Actual concentration of each lipid in linearity solution preparation						
Lipids	level 1	level 2	level 3	level 4	level 5	level 6
PEG2000DMG (6548)	s47					
Cholesterol (6546)	s47					
SM102 (6547)	s47					
DSPC (6542)	s47					

ASSAY CONTROL PREPARATION DETAILS

Date control solution prepared: 16 Aug 2021 Within expiry Y/N-YES

Attached or Refer to (TRIM/ Record number/Sample number) D21-3106881

Preparation	Sample solution (uL)	EtOH (uL)	DF
1	500	500	2

SAMPLE PREPARATION DETAILS

Date prepared: 18 Sep 2021 Prepared by: s22 POVA LIMS: 33271

Preparation	Sample solution (uL)	EtOH (uL)	DF
1	250	750	4
2	250	750	4

Ethanol make : Mreck

B. No. K52687883035

Signature of Analyst

Signed electronically s22

Date 19 Sep 2021

Checked by Official Analyst...Signed electronically by s22Date...19/09/21...



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Laboratories Branch

Department of Health

Therapeutic Goods Administration

Owner: s22	Number: Chem-Form-6
Author: s22	Version: 1
Active: 27/06/2021	Review: <QPulse_DocReviewDate>
Title: Analysts notes - Worksheet	

ANALYSTS' NOTES**Sample Name** Spikevax (Moderna)**TGA Sample No.** 2109003344**Mobile phase preparation:**

Accurately weighed 3.8556 g of ammonium acetate (Sigma, Lichropur eluent additive for LC-MS, Lot # BCCB9847) and transferred into 400 mL ultrapure water from Sartorius (LIMS 33031) then added 7.5 mL of acetic acid (Merck, Lichropur LC-MS, Lot # Z0656301 027) stirred to dissolve and measured the solution pH as 4.2 using pH meter (LIMS 32688). Added ultrapure water to make 500 mL, filtered using 0.2µm Nylon filter paper. Prepared on 02 Sep 2021.

Mobile phase A: 5 mM ammonium acetate buffer solution, pH 4.2

- Diluted 50 mL of 100 mM ammonium acetate buffer solution pH 4.2 to 1000 mL with ultrapure water. Prepared on 02 Sep 2021

Mobile phase B: 5 mM ammonium acetate/Isopropanol/Acetonitrile (5: 62: 33)

- Mixed 250 mL of 100 mM ammonium acetate buffer solution pH 4.2 with 3100 mL of isopropanol and 1650 mL of acetonitrile. Prepared on 17 Sep 2021.

Seal wash: 10% Acetonitrile. Prepared on 17 Sep 2021.

Needle wash: 90% MeOH. Prepared on 17 Sep 2021.

Column flush: 100% Acetonitrile

Chemicals used in mobile phase preparation:

Ammonium acetate – Sigma, Lichropur eluent additive for LC-MS, Lot # BCCB9847.

Acetic acid - Merck, Lichropur LC-MS, Lot # Z0656301 027

IPA – Fisher – Optima LC-MS grade, Lot # 200525

ACN – Merck – Gradient grade, Lot # I1135530106

MeOH – Fisher – Optima, LC-MS grade, Lot # 205074

Signature of Analyst Electronically signed s22 Date 19 Sep 2021

Checked by Official Analyst... Electronically signed by s22 Date... 19/09/21...



Australian Government

Department of Health

Therapeutic Goods Administration

Laboratories Branch

Owner: s22	Number: Chem-Form-6
Author: s22	Version: 1
Active: 27/06/2021	Review: <QPulse_DocReviewDate>
Title: Analysts notes - Worksheet	

ANALYSTS' NOTES**Sample Name** Spikevax (Moderna)**TGA Sample No.** 2109003344**Resolution standard solution preparation:**

Preparation of 1 mg/mL lyso-PC solution: 1.067 mg of lyso-PC weighed and dissolved in 1 mL EtOH. The lyso-PC solution contains lyso-PC isomer.

Note: Lyso-PC weighed lower than the balance minimum weight capacity (2 mg) – No impact on results as lyso-PC is included in the resolution standard solution only for identification.

Preparation of 4 mg/mL stearic acid solution: 4.025 mg of stearic acid weighed and dissolved in 1 mL EtOH.

Add components listed in the table 6 below and mix well.

Each component in the resolution standard solution

Component	Volume μ L
1 mg/mL lyco-PC	25
4 mg/mL stearic acid	25
Mixed lipid stock solution (250%)	400
Ethanol	550

The final resolution standard solution contains: lyso-PC, lyso-PC isomer, PEG 2000 DMG, stearic acid, cholesterol, SM102 and DSPC.

Lyco-PC and stearic acid stock solutions were prepared on 29 Jul 2021 – refer to validation data.
Mixed lipid stock solution was prepared on 26 Aug 2021.

Ethanol make : Merck B. No. K52687883035

LIMS POVA: 33271

Signature of Analyst Signed electronically s22 Date 19 Sep 2021

Checked by Official Analyst...Signed electronically by s22Date...19/09/21...

Vaccine System Suitability Report

No blank peak should appear within the retention time window of the lipid peaks (RT 4 to 20 mins) Pass / ~~Fail~~

The %RSD of the peak area and retention time of the first 3 SST injections must be NMT 5.0% Pass / ~~Fail~~

Peak Results Retention Time Summarized by Name						Peak Results Area Summarized by Name					
	Result Id	PEG2000 DMG (min)	Cholesterol (min)	SM102 (min)	DSPC (min)	Result Id	PEG2000 DMG (μV*sec)	Cholesterol (μV*sec)	SM102 (μV*sec)	DSPC (μV*sec)	
1	10939	5.161	10.006	13.365	17.979	1	10939	s47			
2	10941	5.118	10.002	13.355	17.963	2	10941				
3	10943	5.155	9.982	13.334	17.941	3	10943				
Mean		5.145	9.997	13.351	17.961	Mean					
% RSD		0.46	0.13	0.12	0.11	% RSD					0.51

The %RSD of the peak area and retention time of the all 5 SST injections must be NMT 5.0% Pass / ~~Fail~~

Peak Results Retention Time Summarized by Name						Peak Results Area Summarized by Name					
	Result Id	PEG2000 DMG (min)	Cholesterol (min)	SM102 (min)	DSPC (min)	Result Id	PEG2000 DMG (μV*sec)	Cholesterol (μV*sec)	SM102 (μV*sec)	DSPC (μV*sec)	
1	10941	5.118	10.002	13.355	17.963	1	10941	s47			
2	10945	5.149	9.984	13.400	17.927	2	10945				
3	10943	5.155	9.982	13.334	17.941	3	10943				
4	10939	5.161	10.006	13.365	17.979	4	10939				
5	10947	5.167	9.983	13.403	17.937	5	10947				
Mean		5.150	9.991	13.371	17.950	Mean					
% RSD		0.38	0.12	0.22	0.12	% RSD		0.92	0.87	1.34	2.21

The % Recovery of the lipids in the assay control must be within 85% - 115% (% Deviation within ±15%) Pass / ~~Fail~~

Peak Results Percent_LipidRecovery Summarized by Name					
	Result Id	PEG2000 DMG	Cholesterol	SM102	DSPC
1	10949	89.440	100.826	102.180	100.573
2	10951	89.111	100.275	101.778	99.729
Mean		89.275	100.550	101.979	100.151

Project Name: Chemistry\System20_2021_05_Lipids

Sample Set Name: 20210918 Moderna Vac testing

Sample Set Acquired By **s22**

Sample LIMS No. 2109003344 **Document 4**

Sample Set Id: 10717

Sample Set Start Date: 18/09/2021 12:53:22 PM AEST

Print Date: 19/09/2021

Vaccine System Suitability Report

The % RT difference between sample preparations and SST must be within
95% - 105% (% Deviation within $\pm 5\%$)

Pass / ~~Fail~~

Peak Results
RT_Dev Summarized by Name

	SampleName	Result Id	PEG2000 DMG	Cholesterol	SM102	DSPC
1	2109003344 prep 1	10953	100.932	100.081	100.513	99.991
2	2109003344 prep 1	10955	100.932	100.081	100.513	99.991
3	2109003344 prep 2	10957	100.932	100.081	100.513	99.991
4	2109003344 prep 2	10959	100.932	100.081	100.513	99.991
Mean			100.9	100.1	100.5	100.0

Vaccine System Suitability Report

The R2 values for the Cal Curves must be greater than 0.995

Pass / ~~Fail~~

Peak: SM102

	Name	Level	X Value	Response	Calc. Value	% Deviation	Manual	Ignore	R^2
1	SM102	Level 1	s47			-1.511	No	No	0.999945
2	SM102	Level 1				-0.538	No	No	0.999945
3	SM102	Level 2				0.326	No	No	0.999945
4	SM102	Level 2				-0.114	No	No	0.999945
5	SM102	Level 3				0.477	No	No	0.999945
6	SM102	Level 3				0.575	No	No	0.999945
7	SM102	Level 4				0.065	No	No	0.999945
8	SM102	Level 4				0.301	No	No	0.999945
9	SM102	Level 5				-0.662	No	No	0.999945
10	SM102	Level 5				-0.539	No	No	0.999945
11	SM102	Level 6				0.275	No	No	0.999945
12	SM102	Level 6				0.210	No	No	0.999945
Mean									1.0

Peak: DSPC

	Name	Level	X Value	Response	Calc. Value	% Deviation	Manual	Ignore	R^2
1	DSPC	Level 1	s47			0.325	No	No	0.999970
2	DSPC	Level 1				1.311	No	No	0.999970
3	DSPC	Level 2				0.422	No	No	0.999970
4	DSPC	Level 2				-1.553	No	No	0.999970
5	DSPC	Level 3				-0.009	No	No	0.999970
6	DSPC	Level 3				-0.245	No	No	0.999970
7	DSPC	Level 4				0.235	No	No	0.999970
8	DSPC	Level 4				0.363	No	No	0.999970
9	DSPC	Level 5				-0.041	No	No	0.999970
10	DSPC	Level 5				-0.261	No	No	0.999970
11	DSPC	Level 6				-0.250	No	No	0.999970
12	DSPC	Level 6				0.297	No	No	0.999970
Mean									1.0

Peak: PEG2000 DMG

	Name	Level	X Value	Response	Calc. Value	% Deviation	Manual	Ignore	R^2
1	PEG2000 DMG	Level 1	s47			-0.911	No	No	0.999983
2	PEG2000 DMG	Level 1				-1.281	No	No	0.999983
3	PEG2000 DMG	Level 2				0.335	No	No	0.999983
4	PEG2000 DMG	Level 2				1.291	No	No	0.999983
5	PEG2000 DMG	Level 3				-0.229	No	No	0.999983
6	PEG2000 DMG	Level 3				-0.050	No	No	0.999983
7	PEG2000 DMG	Level 4				0.279	No	No	0.999983

Vaccine System Suitability Report

Peak: PEG2000 DMG

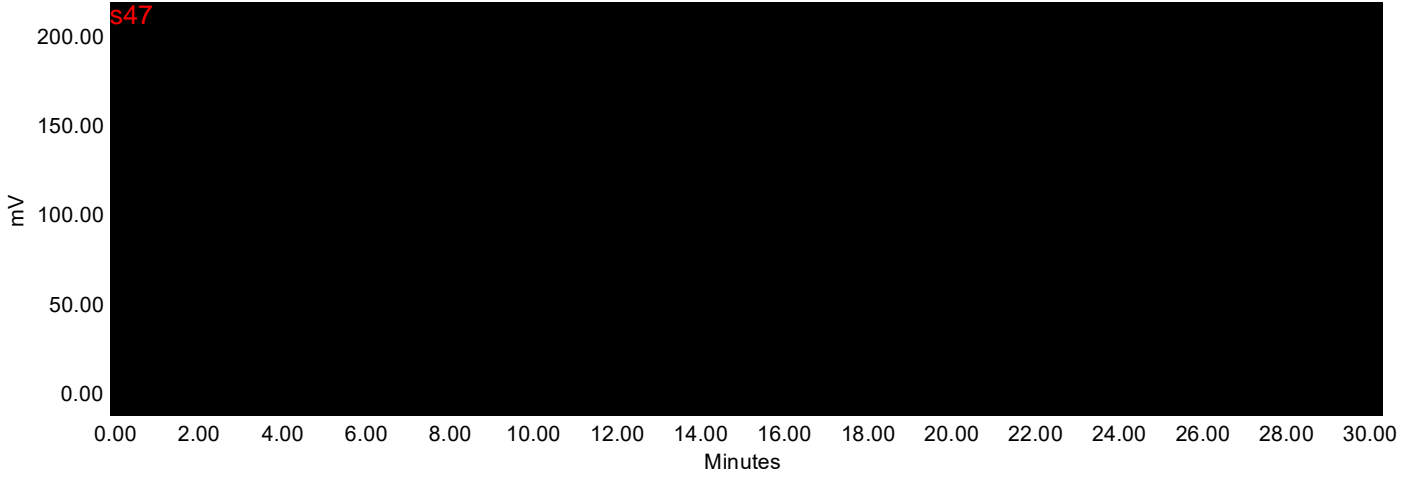
	Name	Level	X Value	Response	Calc. Value	% Deviation	Manual	Ignore	R^2
8	PEG2000 DMG	Level 4	s47			-0.059	No	No	0.999983
9	PEG2000 DMG	Level 5				-0.012	No	No	0.999983
10	PEG2000 DMG	Level 5				-0.294	No	No	0.999983
11	PEG2000 DMG	Level 6				0.054	No	No	0.999983
12	PEG2000 DMG	Level 6				0.064	No	No	0.999983
Mean									1.0

Peak: Cholesterol

	Name	Level	X Value	Response	Calc. Value	% Deviation	Manual	Ignore	R^2
1	Cholesterol	Level 1	s47			-1.026	No	No	0.999859
2	Cholesterol	Level 1				-1.187	No	No	0.999859
3	Cholesterol	Level 2				-0.331	No	No	0.999859
4	Cholesterol	Level 2				-0.104	No	No	0.999859
5	Cholesterol	Level 3				0.433	No	No	0.999859
6	Cholesterol	Level 3				1.475	No	No	0.999859
7	Cholesterol	Level 4				-0.354	No	No	0.999859
8	Cholesterol	Level 4				0.666	No	No	0.999859
9	Cholesterol	Level 5				-0.630	No	No	0.999859
10	Cholesterol	Level 5				-1.052	No	No	0.999859
11	Cholesterol	Level 6				0.598	No	No	0.999859
12	Cholesterol	Level 6				0.111	No	No	0.999859
Mean									1.0

Example Chromatograms

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	USP Resolution
1	Lyso PC	3.677	s47		
2	PEG2000DMG	5.156			2.32
3	Stearic acid	5.993			1.31
4	Cholesterol	10.008			18.03
5	SM102	13.353			5.43
6	DSPC	17.984			7.06

Project Name: Chemistry\System20_2021_05_Lipids

Sample Set Name: 20210918 Moderna Vac testing

Sample Set Acquired By: s22

Sample LIMS No. 2109003344 Document 4

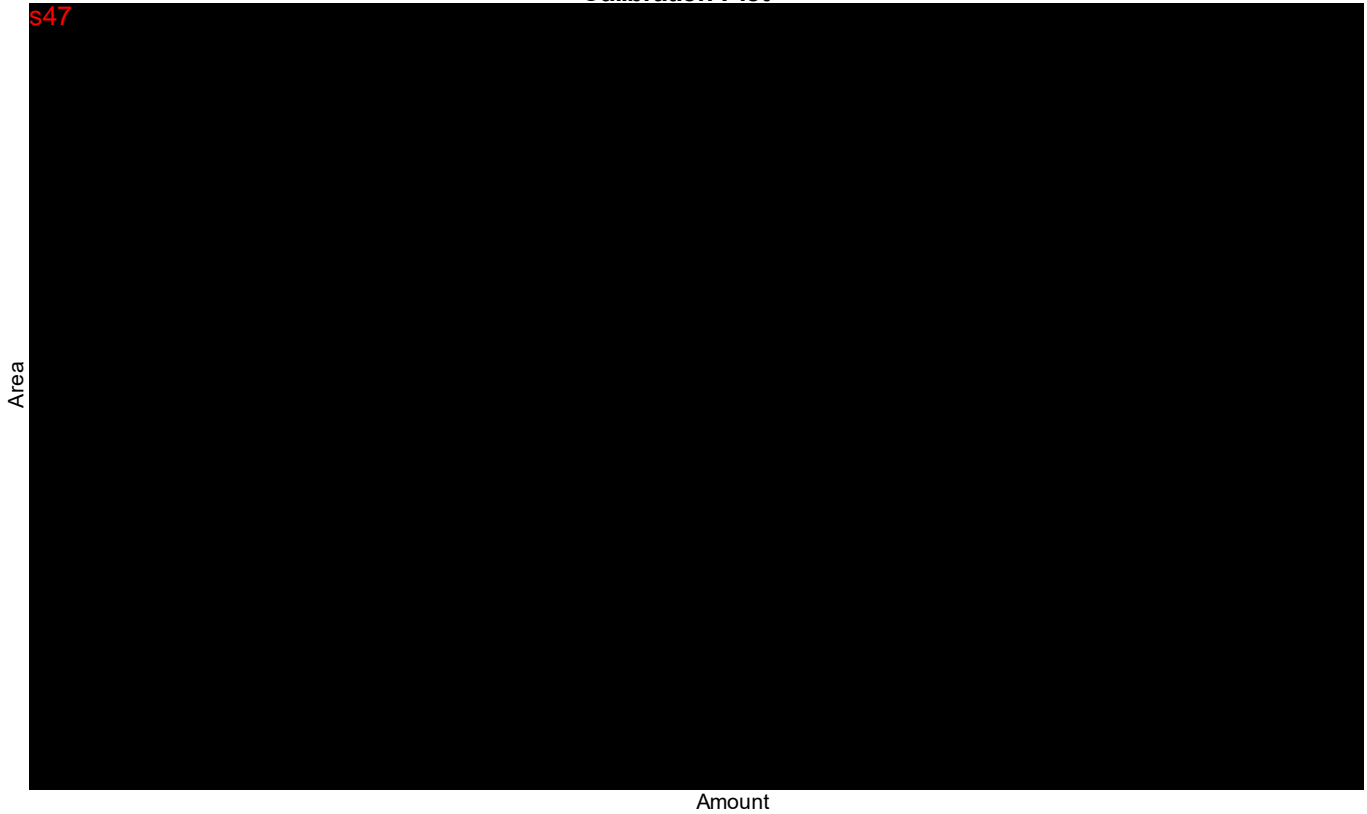
Sample Set Id: 10717

Sample Set Start Date: 18/09/2021 12:53:22 PM AEST

Print Date: 19/09/2021

Cal Curve Report

Calibration Plot



Name: PEG2000 DMG; Processing Method: Moderna; Fit Type: Quadratic (2nd Order); Cal Curve Id: 10891; A: 3.873007e+04; B: 1.095099e+07; C: -4.442572e+06; D: 0.000000e+00; R^2: 0.999983

Project Name: Chemistry\System20_2021_05_Lipids

Sample Set Name: 20210918 Moderna Vac testing

Sample Set Acquired By: s22

Sample LIMS No. 2109003344 Document 4

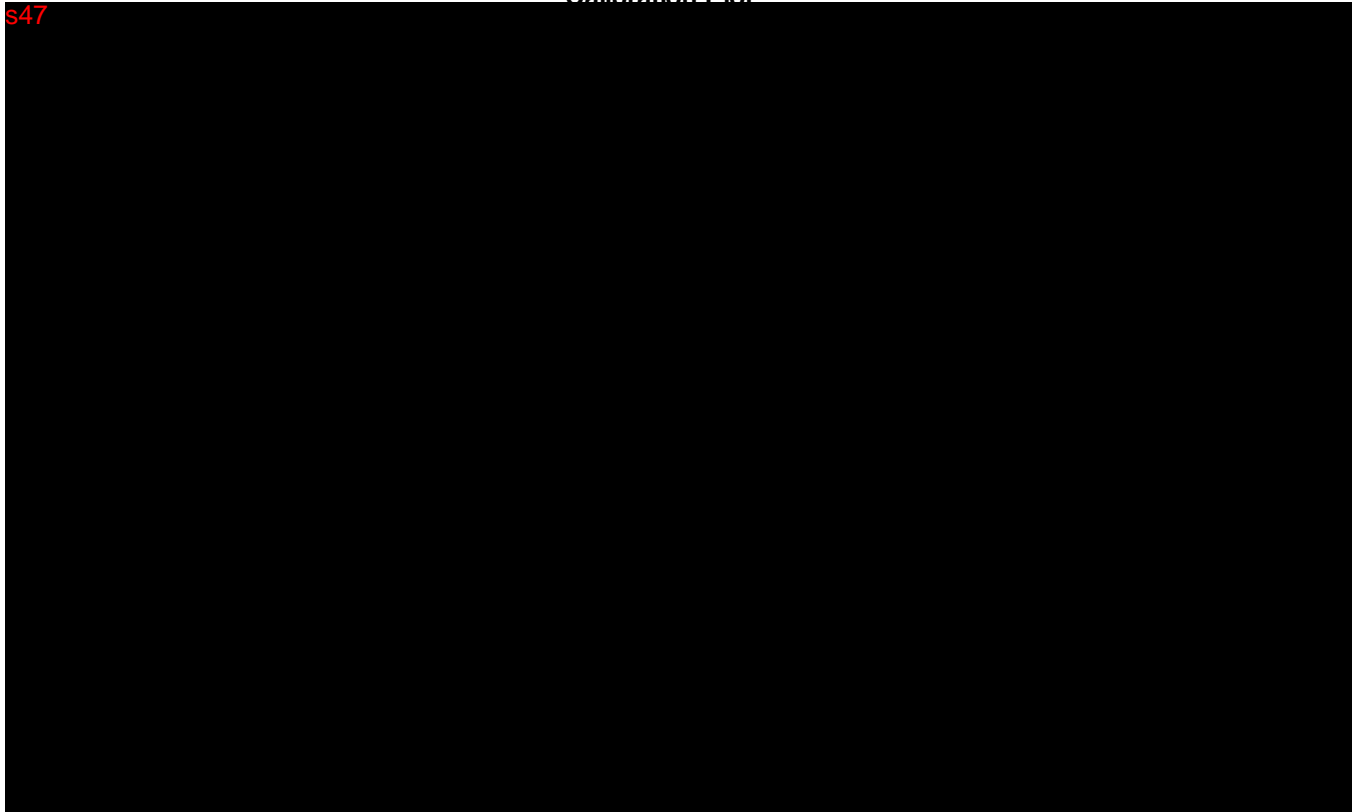
Sample Set Id: 10717

Sample Set Start Date: 18/09/2021 12:53:22 PM AEST

Print Date: 19/09/2021

Cal Curve Report

Calibration Plot



Name: Cholesterol; Processing Method: Moderna; Fit Type: Quadratic (2nd Order); Cal Curve Id: 10892; A: -1.582455e+04; B: 8.868176e+06; C: -2.057060e+06; D: 0.000000e+00; R^2: 0.999859

Project Name: Chemistry\System20_2021_05_Lipids

Sample Set Name: 20210918 Moderna Vac testing

Sample Set Acquired By: s22

Sample LIMS No. 2109003344 Document 4

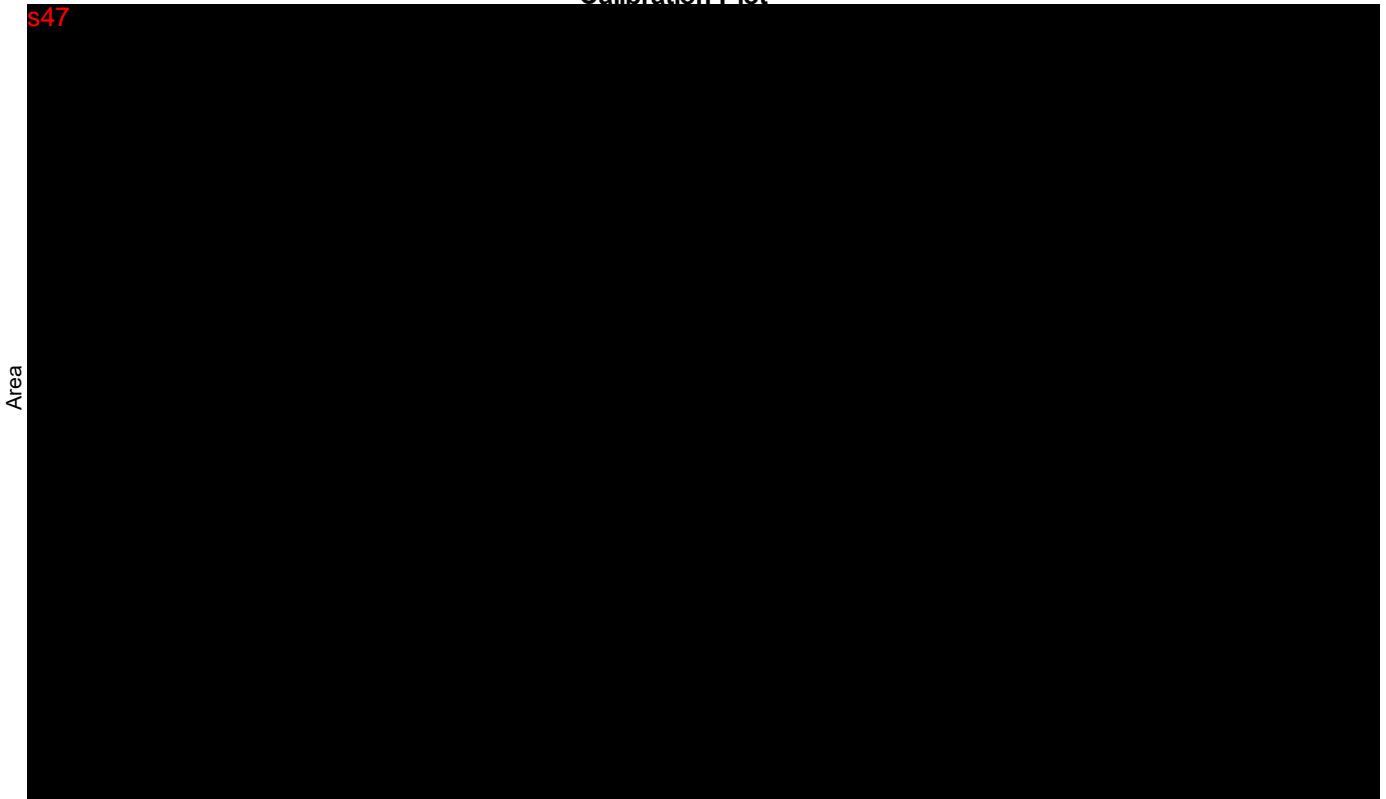
Sample Set Id: 10717

Sample Set Start Date: 18/09/2021 12:53:22 PM AEST

Print Date: 19/09/2021

Cal Curve Report

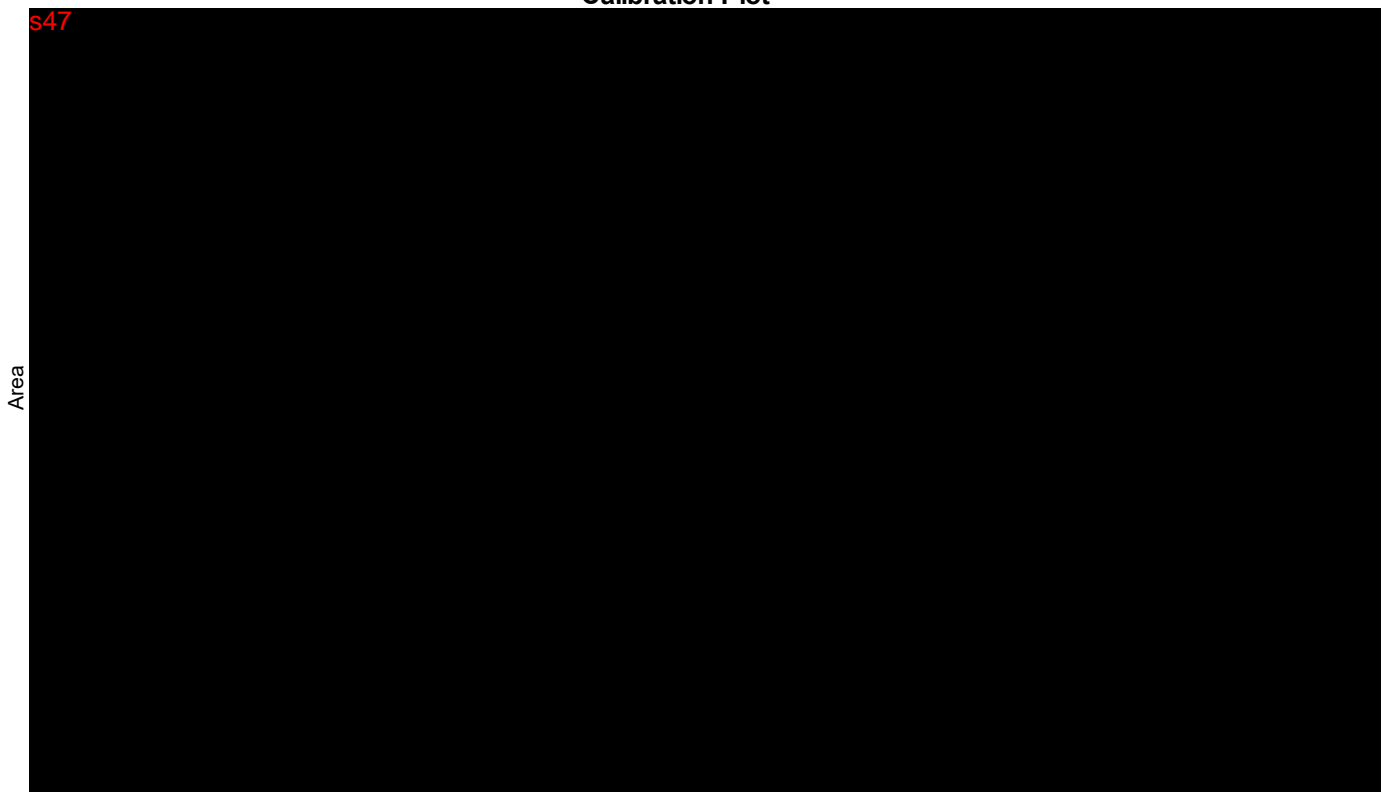
Calibration Plot



Name: SM102; Processing Method: Moderna; Fit Type: Quadratic (2nd Order); Cal Curve Id: 10893;
A: 1.826920e+05; B: 8.891164e+06; C: -6.909508e+05; D: 0.000000e+00; R²: 0.999945

Cal Curve Report

Calibration Plot



Amount

Name: DSPC; Processing Method: Moderna; Fit Type: Quadratic (2nd Order); Cal Curve Id: 10894; A: 1.447778e+04; B: 1.008808e+07; C: -4.847926e+06; D: 0.000000e+00; R^2: 0.999970

Peak: SM102

	Name	Level	X Value	Response	Calc. Value	% Deviation	Manual	Ignore	R^2
1	SM102	Level 1	s47			-1.511	No	No	0.999945
2	SM102	Level 1				-0.538	No	No	0.999945
3	SM102	Level 2				0.326	No	No	0.999945
4	SM102	Level 2				-0.114	No	No	0.999945
5	SM102	Level 3				0.477	No	No	0.999945
6	SM102	Level 3				0.575	No	No	0.999945
7	SM102	Level 4				0.065	No	No	0.999945
8	SM102	Level 4				0.301	No	No	0.999945
9	SM102	Level 5				-0.662	No	No	0.999945
10	SM102	Level 5				-0.539	No	No	0.999945
11	SM102	Level 6				0.275	No	No	0.999945
12	SM102	Level 6				0.210	No	No	0.999945

Cal Curve Report

Peak: DSPC

	Name	Level	X Value	Response	Calc. Value	% Deviation	Manual	Ignore	R^2
1	DSPC	Level 1	s47			0.325	No	No	0.999970
2	DSPC	Level 1				1.311	No	No	0.999970
3	DSPC	Level 2				0.422	No	No	0.999970
4	DSPC	Level 2				-1.553	No	No	0.999970
5	DSPC	Level 3				-0.009	No	No	0.999970
6	DSPC	Level 3				-0.245	No	No	0.999970
7	DSPC	Level 4				0.235	No	No	0.999970
8	DSPC	Level 4				0.363	No	No	0.999970
9	DSPC	Level 5				-0.041	No	No	0.999970
10	DSPC	Level 5				-0.261	No	No	0.999970
11	DSPC	Level 6				-0.250	No	No	0.999970
12	DSPC	Level 6				0.297	No	No	0.999970

Peak: PEG2000 DMG

	Name	Level	X Value	Response	Calc. Value	% Deviation	Manual	Ignore	R^2
1	PEG2000 DMG	Level 1	s47			-0.911	No	No	0.999983
2	PEG2000 DMG	Level 1				-1.281	No	No	0.999983
3	PEG2000 DMG	Level 2				0.335	No	No	0.999983
4	PEG2000 DMG	Level 2				1.291	No	No	0.999983
5	PEG2000 DMG	Level 3				-0.229	No	No	0.999983
6	PEG2000 DMG	Level 3				-0.050	No	No	0.999983
7	PEG2000 DMG	Level 4				0.279	No	No	0.999983
8	PEG2000 DMG	Level 4				-0.059	No	No	0.999983
9	PEG2000 DMG	Level 5				-0.012	No	No	0.999983
10	PEG2000 DMG	Level 5				-0.294	No	No	0.999983
11	PEG2000 DMG	Level 6				0.054	No	No	0.999983
12	PEG2000 DMG	Level 6				0.064	No	No	0.999983

Peak: Cholesterol

	Name	Level	X Value	Response	Calc. Value	% Deviation	Manual	Ignore	R^2
1	Cholesterol	Level 1	s47			-1.026	No	No	0.999859
2	Cholesterol	Level 1				-1.187	No	No	0.999859
3	Cholesterol	Level 2				-0.331	No	No	0.999859
4	Cholesterol	Level 2				-0.104	No	No	0.999859
5	Cholesterol	Level 3				0.433	No	No	0.999859
6	Cholesterol	Level 3				1.475	No	No	0.999859
7	Cholesterol	Level 4				-0.354	No	No	0.999859
8	Cholesterol	Level 4				0.666	No	No	0.999859

Project Name: Chemistry\System20_2021_05_Lipids

Sample Set Name: 20210918 Moderna Vac testing

Sample Set Acquired By **s22**

Sample LIMS No. 2109003344 **Document 4**

Sample Set Id: 10717

Sample Set Start Date: 18/09/2021 12:53:22 PM AEST

Print Date: 19/09/2021

Cal Curve Report

Peak: Cholesterol

	Name	Level	X Value	Response	Calc. Value	% Deviation	Manual	Ignore	R^2
9	Cholesterol	Level 5	s47			-0.630	No	No	0.999859
10	Cholesterol	Level 5				-1.052	No	No	0.999859
11	Cholesterol	Level 6				0.598	No	No	0.999859
12	Cholesterol	Level 6				0.111	No	No	0.999859

Result Summary Report

Result Summary Table (peak area)
Ave_Conc Summarized by Name

	Inj Id	Result Id	Sample Name	PEG2000 DMG	Cholesterol	SM102	DSPC
1	10798	10953	2109003344 prep 1	s47			
2	10805	10957	2109003344 prep 2				
Mean							
Std. Dev.							
% RSD				1.5	0.7	0.3	0.1

Result Summary Table (peak area)
Amount Summarized by Name

	Inj Id	Result Id	Sample Name	PEG2000 DMG	Cholesterol	SM102	DSPC
1	10798	10953	2109003344 prep 1	s47			
2	10801	10955	2109003344 prep 1				
3	10805	10957	2109003344 prep 2				
4	10808	10959	2109003344 prep 2				
Mean				0.20	0.95	2.10	0.54

Result Summary Table (peak area)
Amount Summarized by Name

	Inj Id	Result Id	Sample Name	PEG2000 DMG	Cholesterol	SM102	DSPC
1	10784	10949	Assay control	s47			
2	10787	10951	Assay control				
Mean							

Results Summary Report

Calibration Data Table

Peak: SM102

	Inj Id	Result Id	Name	Concentration	Response	Cal Curve Id
1	10740	10901	SM102	s47		10893
2	10743	10903	SM102			10893
3	10749	10905	SM102			10893
4	10752	10907	SM102			10893
5	10756	10909	SM102			10893
6	10759	10911	SM102			10893
7	10763	10913	SM102			10893
8	10766	10915	SM102			10893
9	10770	10917	SM102			10893
10	10773	10919	SM102			10893
11	10777	10921	SM102			10893
12	10780	10923	SM102			10893

Calibration Data Table

Peak: DSPC

	Inj Id	Result Id	Name	Concentration	Response	Cal Curve Id
1	10740	10901	DSPC	s47		10894
2	10743	10903	DSPC			10894
3	10749	10905	DSPC			10894
4	10752	10907	DSPC			10894
5	10756	10909	DSPC			10894
6	10759	10911	DSPC			10894
7	10763	10913	DSPC			10894
8	10766	10915	DSPC			10894
9	10770	10917	DSPC			10894
10	10773	10919	DSPC			10894
11	10777	10921	DSPC			10894
12	10780	10923	DSPC			10894

Calibration Data Table

Peak: PEG2000 DMG

	Inj Id	Result Id	Name	Concentration	Response	Cal Curve Id
1	10740	10901	PEG2000 DMG	s47		10891
2	10743	10903	PEG2000 DMG			10891
3	10749	10905	PEG2000 DMG			10891
4	10752	10907	PEG2000 DMG			10891
5	10756	10909	PEG2000 DMG			10891
6	10759	10911	PEG2000 DMG			10891
7	10763	10913	PEG2000 DMG			10891
8	10766	10915	PEG2000 DMG			10891
9	10770	10917	PEG2000 DMG			10891
10	10773	10919	PEG2000 DMG			10891

Results Summary Report

Calibration Data Table

Peak: PEG2000 DMG

	Inj Id	Result Id	Name	Concentration	Response	Cal Curve Id
11	10777	10921	PEG2000 DMG	s47		10891
12	10780	10923	PEG2000 DMG			10891

Calibration Data Table

Peak: Cholesterol

	Inj Id	Result Id	Name	Concentration	Response	Cal Curve Id
1	10740	10901	Cholesterol	s47		10892
2	10743	10903	Cholesterol			10892
3	10749	10905	Cholesterol			10892
4	10752	10907	Cholesterol			10892
5	10756	10909	Cholesterol			10892
6	10759	10911	Cholesterol			10892
7	10763	10913	Cholesterol			10892
8	10766	10915	Cholesterol			10892
9	10770	10917	Cholesterol			10892
10	10773	10919	Cholesterol			10892
11	10777	10921	Cholesterol			10892
12	10780	10923	Cholesterol			10892

Results Summary Report

Result Summary Table (peak area)
Peak Name: Cholesterol

	Inj Id	Result Id	Sample Name	RT	Area ($\mu\text{V}\cdot\text{sec}$)	Height (μV)	Lipid Content
1	10730	10939	SST	10.006	s47		
2	10733	10941	SST	10.002			
3	10736	10943	SST	9.982			
4	10740	10901	LEVEL 1 25%	9.989			
5	10743	10903	LEVEL 1 25%	9.985			
6	10749	10905	LEVEL 2 50%	9.983			
7	10752	10907	LEVEL 2 50%	9.993			
8	10756	10909	LEVEL 3 100%	9.986			
9	10759	10911	LEVEL 3 100%	9.978			
10	10763	10913	LEVEL 4 150%	9.982			
11	10766	10915	LEVEL 4 150%	9.983			
12	10770	10917	LEVEL 5 200%	9.980			
13	10773	10919	LEVEL 5 200%	9.980			
14	10777	10921	LEVEL 6 250%	9.979			
15	10780	10923	LEVEL 6 250%	9.999			
16	10784	10949	Assay control	9.985			
17	10787	10951	Assay control	9.981			
18	10798	10953	2109003344 prep 1	9.995			
19	10801	10955	2109003344 prep 1	10.000			
20	10805	10957	2109003344 prep 2	10.000			
21	10808	10959	2109003344 prep 2	10.003			
22	10816	10945	SST	9.984			
23	10819	10947	SST	9.983			

Result Summary Table (peak area)
Peak Name: DSPC

	Inj Id	Result Id	Sample Name	RT	Area ($\mu\text{V}\cdot\text{sec}$)	Height (μV)	Lipid Content
1	10730	10939	SST	17.979	s47		
2	10733	10941	SST	17.963			
3	10736	10943	SST	17.941			
4	10740	10901	LEVEL 1 25%	17.935			
5	10743	10903	LEVEL 1 25%	17.928			
6	10749	10905	LEVEL 2 50%	17.932			
7	10752	10907	LEVEL 2 50%	17.946			
8	10756	10909	LEVEL 3 100%	17.922			
9	10759	10911	LEVEL 3 100%	17.910			
10	10763	10913	LEVEL 4 150%	17.929			
11	10766	10915	LEVEL 4 150%	17.920			
12	10770	10917	LEVEL 5 200%	17.926			
13	10773	10919	LEVEL 5 200%	17.935			
14	10777	10921	LEVEL 6 250%	17.931			
15	10780	10923	LEVEL 6 250%	17.959			

Analyst s22

Results Summary Report

Result Summary Table (peak area)
 Peak Name: DSPC

	Inj Id	Result Id	Sample Name	RT	Area (μV*sec)	Height (μV)	Lipid Content
16	10784	10949	Assay control	17.935	s47		
17	10787	10951	Assay control	17.928			
18	10798	10953	2109003344 prep 1	17.940			
19	10801	10955	2109003344 prep 1	17.945			
20	10805	10957	2109003344 prep 2	17.958			
21	10808	10959	2109003344 prep 2	17.950			
22	10816	10945	SST	17.927			
23	10819	10947	SST	17.937			

Result Summary Table (peak area)
 Peak Name: PEG2000 DMG

	Inj Id	Result Id	Sample Name	RT	Area (μV*sec)	Height (μV)	Lipid Content
1	10730	10939	SST	5.161	s47		
2	10733	10941	SST	5.118			
3	10736	10943	SST	5.155			
4	10740	10901	LEVEL 1 25%	5.199			
5	10743	10903	LEVEL 1 25%	5.166			
6	10749	10905	LEVEL 2 50%	5.114			
7	10752	10907	LEVEL 2 50%	5.141			
8	10756	10909	LEVEL 3 100%	5.142			
9	10759	10911	LEVEL 3 100%	5.126			
10	10763	10913	LEVEL 4 150%	5.142			
11	10766	10915	LEVEL 4 150%	5.164			
12	10770	10917	LEVEL 5 200%	5.100			
13	10773	10919	LEVEL 5 200%	5.153			
14	10777	10921	LEVEL 6 250%	5.111			
15	10780	10923	LEVEL 6 250%	5.148			
16	10784	10949	Assay control	5.157			
17	10787	10951	Assay control	5.161			
18	10798	10953	2109003344 prep 1	5.224			
19	10801	10955	2109003344 prep 1	5.205			
20	10805	10957	2109003344 prep 2	5.193			
21	10808	10959	2109003344 prep 2	5.170			
22	10816	10945	SST	5.149			
23	10819	10947	SST	5.167			

Result Summary Table (peak area)
 Peak Name: SM102

	Inj Id	Result Id	Sample Name	RT	Area (μV*sec)	Height (μV)	Lipid Content
1	10730	10939	SST	13.365	s47		
2	10733	10941	SST	13.355			
3	10736	10943	SST	13.334			
4	10740	10901	LEVEL 1 25%	13.759			
5	10743	10903	LEVEL 1 25%	13.752			

Results Summary Report

Result Summary Table (peak area)

Peak Name: SM102

	Inj Id	Result Id	Sample Name	RT	Area ($\mu\text{V}\cdot\text{sec}$)	Height (μV)	Lipid Content
6	10749	10905	LEVEL 2 50%	13.575	s47		
7	10752	10907	LEVEL 2 50%	13.598			
8	10756	10909	LEVEL 3 100%	13.356			
9	10759	10911	LEVEL 3 100%	13.337			
10	10763	10913	LEVEL 4 150%	13.186			
11	10766	10915	LEVEL 4 150%	13.182			
12	10770	10917	LEVEL 5 200%	13.045			
13	10773	10919	LEVEL 5 200%	13.036			
14	10777	10921	LEVEL 6 250%	12.913			
15	10780	10923	LEVEL 6 250%	12.943			
16	10784	10949	Assay control	13.247			
17	10787	10951	Assay control	13.247			
18	10798	10953	2109003344 prep 1	13.429			
19	10801	10955	2109003344 prep 1	13.437			
20	10805	10957	2109003344 prep 2	13.457			
21	10808	10959	2109003344 prep 2	13.436			
22	10816	10945	SST	13.400			
23	10819	10947	SST	13.403			

Run and Method Summary Report

	Sample Name	Sample Type	Label	Vial	Inj #	Injection Id	Inj. Vol. (ul)	Dilution	Run Time (Minutes)	Level	Acquisition Method Set
1	EtOH as Blank	Unknown	B101	1:A,1	1	10719	10	1	30		Moderna Vaccine
2	EtOH as Blank	Unknown	B101	1:A,1	2	10722	10	1	30		Moderna Vaccine
3	Resolution std	Unknown		1:A,2	1	10726	10	1	30		Moderna Vaccine
4	SST	Control	Y101	1:A,5	1	10730	10	1	30		Moderna Vaccine
5	SST	Control	Y101	1:A,5	2	10733	10	1	30		Moderna Vaccine
6	SST	Control	Y101	1:A,5	3	10736	10	1	30		Moderna Vaccine
7	LEVEL 1 25%	Standard	S101	1:A,3	1	10740	10	1	30	Level 1	Moderna Vaccine
8	LEVEL 1 25%	Standard	S101	1:A,3	2	10743	10	1	30	Level 1	Moderna Vaccine
9	LEVEL 2 50%	Standard	S102	1:A,4	1	10749	10	1	30	Level 2	Moderna Vaccine
10	LEVEL 2 50%	Standard	S102	1:A,4	2	10752	10	1	30	Level 2	Moderna Vaccine
11	LEVEL 3 100%	Standard	S103	1:A,5	1	10756	10	1	30	Level 3	Moderna Vaccine
12	LEVEL 3 100%	Standard	S103	1:A,5	2	10759	10	1	30	Level 3	Moderna Vaccine
13	LEVEL 4 150%	Standard	S104	1:A,6	1	10763	10	1	30	Level 4	Moderna Vaccine
14	LEVEL 4 150%	Standard	S104	1:A,6	2	10766	10	1	30	Level 4	Moderna Vaccine
15	LEVEL 5 200%	Standard	S105	1:A,7	1	10770	10	1	30	Level 5	Moderna Vaccine
16	LEVEL 5 200%	Standard	S105	1:A,7	2	10773	10	1	30	Level 5	Moderna Vaccine
17	LEVEL 6 250%	Standard	S106	1:A,8	1	10777	10	1	30	Level 6	Moderna Vaccine
18	LEVEL 6 250%	Standard	S106	1:A,8	2	10780	10	1	30	Level 6	Moderna Vaccine
19	Assay control	Unknown	U001	1:B,1	1	10784	10	1	30		Moderna Vaccine
20	Assay control	Unknown	U001	1:B,1	2	10787	10	1	30		Moderna Vaccine
21	EtOH as Blank	Unknown	B102	1:A,1	1	10791	10	1	30		Moderna Vaccine
22	EtOH as Blank	Unknown	B102	1:A,1	2	10794	10	1	30		Moderna Vaccine
23	2109003344 prep 1	Unknown	U101	1:B,2	1	10798	10	4	30		Moderna Vaccine
24	2109003344 prep 1	Unknown	U101	1:B,2	2	10801	10	4	30		Moderna Vaccine
25	2109003344 prep 2	Unknown	U102	1:B,3	1	10805	10	4	30		Moderna Vaccine
26	2109003344 prep 2	Unknown	U102	1:B,3	2	10808	10	4	30		Moderna Vaccine
27	EtOH as Blank	Unknown	B103	1:A,1	1	10812	10	1	30		Moderna Vaccine
28	SST	Control	Y102	1:A,5	1	10816	10	1	30		Moderna Vaccine
29	SST	Control	Y102	1:A,5	2	10819	10	1	30		Moderna Vaccine
30	EtOH as Blank	Unknown	B104	1:A,1	1	10823	10	1	30		Moderna Vaccine
31	EtOH as Blank	Unknown	B104	1:A,1	2	10826	10	1	30		Moderna Vaccine

Column used for analysis: XSelect CSH C18, 3.5µm, 4.6 ×150 mm, S/N: 01533030924070.

Instrument Method: Moderna Vaccine

Stored: 2/09/2021 4:24:39 PM AEST

Method Information

Project Name: Chemistry\System20_2021_05_Lipids

Sample Set Name: 20210918 Moderna Vac testing

Sample Set Acquired By: s22

Sample LIMS No. 2109003344 Document 4

Sample Set Id: 10717

Sample Set Start Date: 18/09/2021 12:53:22 PM AEST

Print Date: 19/09/2021

Run and Method Summary Report

Method Comments: Sampling rate 2, Range 500, Scaling factor 1000, Evop temp 50C
Method Modified User: s22
Method Locked: No
Method Id: 8383
Old Id:
Method Version: 3
Method Edit User:
Source S/W Info: Empower 3 Software Build 3471 SPs Installed: Service Release 5 DB ID: 3107713112

eSATIN Instrument Setup

eSATIN General Information

Environment: 50(Hz)
Enable BCD: Off
BCD Polarity: Positive(+)

Channel 1 Information

Rate: 2.0(Pts/Sec.)
Description: Channel 1
Scale Factor: 1000.0
Units: mV

Channel 2 Information

eSATIN Events Information

Relay Initial State: Off
TTL1 Initial State: Off
TTL2 Initial State: Off
Relay Mode: Standard

ACQ-FTN Instrument Setup

Comment	
Load Ahead	Disabled
Loop Offline	Disabled
Wash Solvent Name	50% MeOH
Pre-Inject Wash Time	0.0(sec)
Post-Inject Wash Time	6.0(sec)
Purge Solvent Name	Water
Dilution	Disabled
Dilution Volume	0(uL)
Delay Time	0(min)
Dilution Needle Placement	4(mm)
Target Column Temperature	40.0(°C)
Column Temperature Alarm Band	Disabled
Target Sample Temperature	20.0(°C)
Sample Temperature Alarm Band	Disabled
Syringe Draw Rate	Automatic
Needle Placement	Automatic
Pre-Aspirate Air Gap	Automatic
Post-Aspirate Air Gap	Automatic
Column Temperature Data Channel	No
Room Temperature Data Channel	No
Sample Temperature Data Channel	No

Project Name: Chemistry\System20_2021_05_Lipids

Sample Set Name: 20210918 Moderna Vac testing

Sample Set Acquired By: s22

Sample LIMS No. 2109003344 Document 4

Sample Set Id: 10717

Sample Set Start Date: 18/09/2021 12:53:22 PM AEST

Print Date: 19/09/2021

Run and Method Summary Report

Sample Organizer Temperature Data Channel No
Sample Pressure Data Channel No
Preheater Temperature Data Channel No
Seal Force Data Channel No
No Injection Mode Enabled No
Autoaddition Mix Stroke Cycles Automatic
Autoaddition Mix Stroke Volume Automatic(uL)
Active Preheater Disabled
Run Events No

ACQ-QSM Instrument Setup

Solvent A Name 5 mM AA pH 4.2
Solvent B Name 5mM AA:IPA:Acetonitrile 5:62:33
Solvent C Name
Solvent D Name
Low Pressure Limit 0(psi)
High Pressure Limit 15000(psi)
Seal Wash Period 5.00(min)

Gradient Table

	Time (min)	Flow Rate (mL/min)	%A	%B	%C	%D	Curve
1	Initial	0.900	15.0	85.0	0.0	0.0	Initial
2	2.00	0.900	15.0	85.0	0.0	0.0	6
3	2.10	0.900	11.0	89.0	0.0	0.0	6
4	15.00	0.900	8.0	92.0	0.0	0.0	6
5	19.00	0.900	0.0	100.0	0.0	0.0	6
6	24.00	0.900	0.0	100.0	0.0	0.0	6
7	26.00	0.900	15.0	85.0	0.0	0.0	6
8	30.00	0.900	15.0	85.0	0.0	0.0	6

Comment

Flow Ramp Rate 0.45(min)
D Solvent Selection (if supported) No Change
System Pressure Data Channel No
Flow Rate Data Channel No
%A Data Channel No
%B Data Channel No
%C Data Channel No
%D Data Channel No
Primary Data Channel No
Accumulator Data Channel No
Degasser Data Channel No
Gradient Start At Injection
Gradient Start Volume 0(uL)
Gradient Start Time 0.00(min)
Participate in pre-analysis No

ACQ-TUV Instrument Setup

Wavelength Mode Single Wavelength
Lamp On On

Project Name: Chemistry\System20_2021_05_Lipids

Sample LIMS No. 2109003344 Document 4

Sample Set Name: 20210918 Moderna Vac testting

Sample Set Id: 10717

Sample Set Acquired By s22

Sample Set Start Date: 18/09/2021 12:53:22 PM AEST

Print Date: 19/09/2021

Run and Method Summary Report

Channel A

Comment
 Wavelength 210(nm)
 Sampling Rate 20(points/sec)
 Data Mode Absorbance
 Time Constant 0.1(sec)
 Autozero On Wavelength Change Maintain Baseline
 Autozero On Inject Start Yes

Analog 1

Sensitivity 2.0000(AUFS)
 Chart Polarity Positive (+)
 Voltage Offset 0(mV)
 Enable Chart Mark Yes

Run Events Yes

Pulse Width 1.0(sec)
 Rect Wave Period 0.2(sec)

Revision History

Version 3 2/09/2021 4:24:39 PM AEST User s22
 Version 2 2/09/2021 2:51:58 PM AEST User s22
 Version 1 17/08/2021 4:37:51 PM AEST User s22 Created method 'Moderna Vaccine'.

Method Version Summaries

	Method Name	Method Type	Method Comments
1	Moderna Vaccine	Instrument	Sampling rate 2, Range 500, Scaling factor 1000, Evop temp 50C
2	Moderna Vaccine	Instrument	Sampling rate 2, Range 500, Scaling factor 1000, Evop temp 50C
3	Moderna Vaccine	Instrument	Sampling rate 2, Range 500, Scaling factor 1000, Evop temp 50C

Method Version Summaries

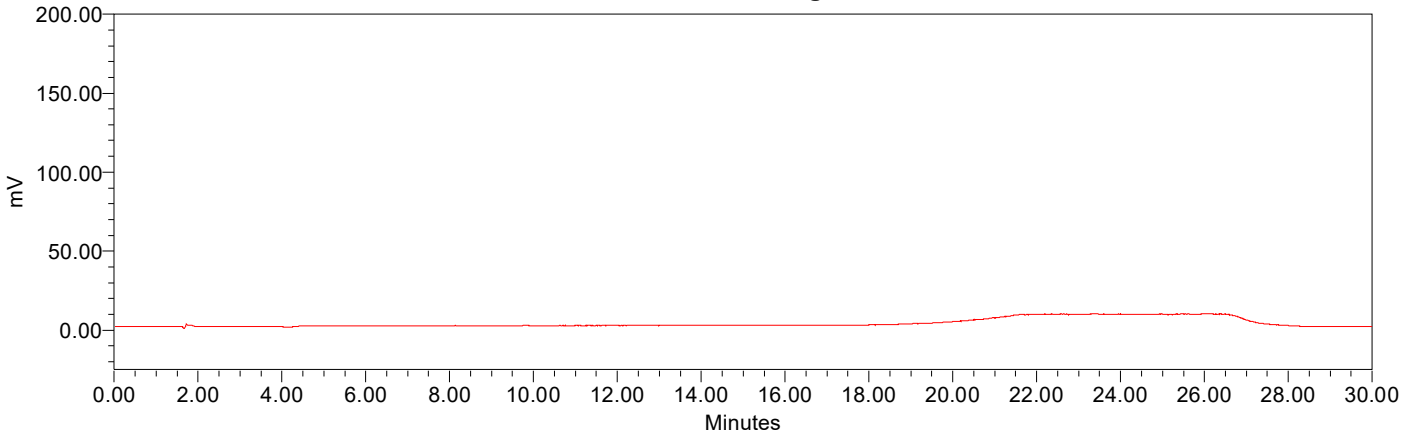
	Method Date	Method Modified User	Method Locked	Method Id	Old Id	Method Version
1	2/09/2021 4:24:39 PM AEST	s22	No	8383		3
2	2/09/2021 2:51:58 PM AEST	s22	No	8376		2
3	17/08/2021 4:37:51 PM AEST	s22	No	6210		1

Method Version Summaries

	Source S/W Info
1	Empower 3 Software Build 3471 SPs Installed: Service Release 5 DB ID: 3107713112
2	Empower 3 Software Build 3471 SPs Installed: Service Release 5 DB ID: 3107713112
3	Empower 3 Software Build 3471 SPs Installed: Service Release 5 DB ID: 3107713112

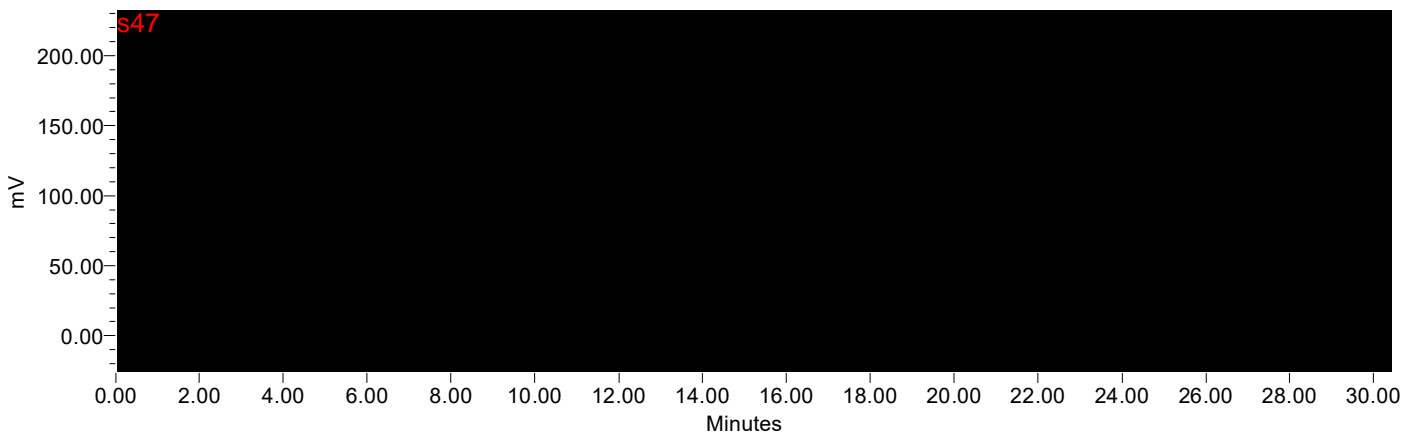
Example Chromatograms

Auto-Scaled Chromatogram



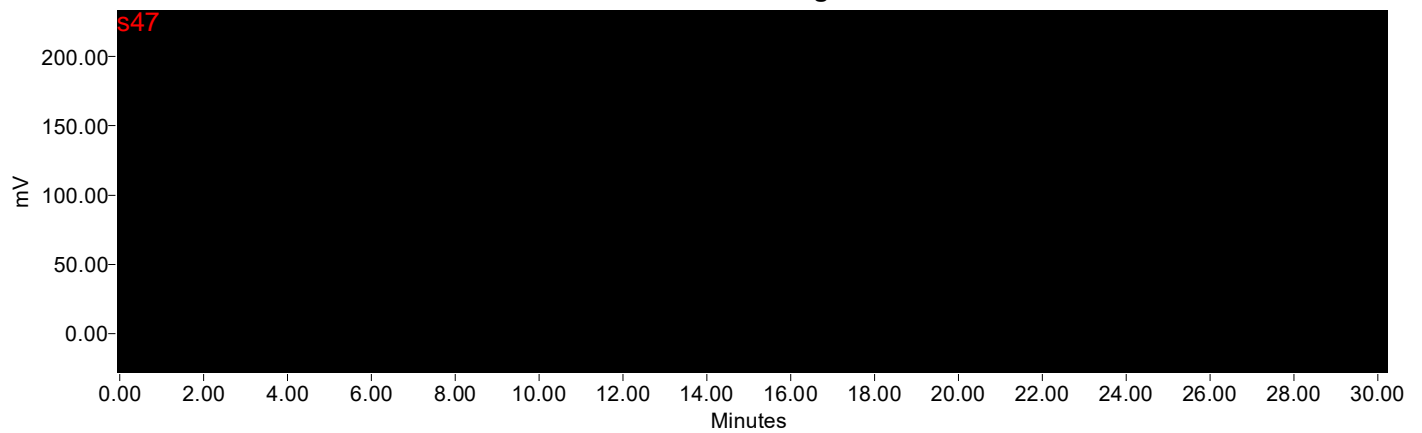
Sample Name EtOH as Blank; Vial 1:A, 1; Injection 2; Injection Id 10722; Channel eSATIN-Ch1; Date Acquired 18/09/2021 1:35:18 PM AEST

Auto-Scaled Chromatogram



Sample Name SST; Vial 1:A,5; Injection 1; Injection Id 10730; Channel eSATIN-Ch1; Date Acquired 18/09/2021 2:37:03 PM AEST

Auto-Scaled Chromatogram



Sample Name 2109003344 prep 1; Vial 1:B,2; Injection 1; Injection Id 10798; Channel eSATIN-Ch1; Date Acquired 19/09/2021 12:23:41 AM AEST

Project Name: Chemistry\System20_2021_05_Lipids

Sample Set Name: 20210918 Moderna Vac testting

Sample Set Acquired By: s22

Sample LIMS No. 2109003344 Document 4

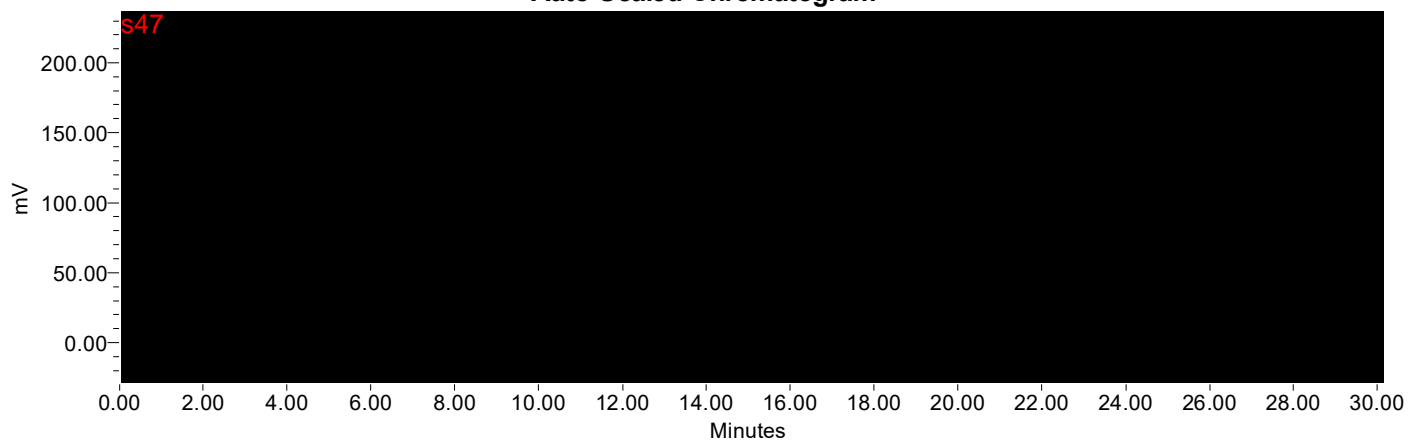
Sample Set Id: 10717

Sample Set Start Date: 18/09/2021 12:53:22 PM AEST

Print Date: 19/09/2021

Example Chromatograms

Auto-Scaled Chromatogram



Sample Name SST; Vial 1:A,5; Injection 2; Injection Id 10819; Channel eSATIN-Ch1; Date Acquired 19/09/2021 3:29:00 AM AEST



Owner: s22	Number: Chem-Method-45
Author: s22	Version: 1
Active: 17/09/2021	Review: 17/03/2023
Title: Identification and Quantitation of PEG 2000 DMG, Cholesterol, SM-102 and DSPC in Moderna Vaccine	

Purpose

The purpose of this method is the identification and quantitation for the found lipids that are part of the COVID-19 vaccine Spikevax. These include: PEG 2000 DMG, cholesterol, SM-102 and DSPC.

Scope

This method applies to drug product (DP) samples with a nominal concentration 0.2 mg/mL mRNA tested in Laboratories Branch at TGA.

Abbreviations

µL	Microlitre
CAD	Charged Aerosol Detection
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
LCMS	Liquid Chromatography Mass Spectrometry
Lyso-PC	1-Stearoyl-sn-glycerol-3-phosphatidylcholine
PEG 2000 DMG	1,2-Dimyristoyl-sn-glycero-3-methoxypolyethylene glycol
RP-UHPLC	Reverse Phase Ultra-High Performance Liquid Chromatography
RT	Retention Time
SM102	Heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate
SST	System suitability
STD	Standard

Method Reference

The method is based on the method for Identification and Quantitation of Lipids in s22 and determination of lipid content, purity and identity by UPLCCAD for SM102/PEG formulations from Moderna.

Equipment, Materials and Reagents

Equipment
Waters Acquity UPLC H-Class equipped with QSM to deliver gradient flow at 0.9 mL/min, SM-FTN, column oven, charged aerosol detector and eSAT/IN.
Waters Xselect CSH, C18 column, 4.6 mm x 150 mm, 3.5 μ m (Waters part No. 186005270)
VanGuard Cartridge Holder (Waters part No. 186007949)
XSelect CSH VanGuard Cartridge, 130 A°, 3.5 μ m x 5 mm (Waters part No. 186007813)
Analytical Balance, capable of reading to 0.1 mg
Centrifuge, capable of 14,000 x g (eg LIMS 32473 (Biotherapeutics))
Sonication Bath
Eppendorf multipette
Vortex
Volumetric flasks
pH meter

Materials
Agilent Glass screw top, high recovery, HPLC vials (part No. 5183-2030) with Waters LCMS certified caps and pre-slit PTFE/Silicone septum (part No. 186005827).
Suitable combitips, recommend 1mL

Reagents
Ultra pure water, resistivity needs to be \Rightarrow 18.2 M Ω (LIMS 33031 is recommended)
Isopropanol (IPA), LCMS Grade
Methanol (MeOH), LCMS Grade
Acetonitrile (ACN), HPLC Grade
Ethanol absolute for analysis (EtOH), ACS Reagent
Ammonium acetate, LCMS Grade
Acetic acid, LCMS Grade
Ammonia solution, LCMS Grade

Mobile phase preparation

100 mM ammonium acetate buffer pH 4.2

Weigh 3.85 g of ammonium acetate into a weight boat, transfer to a 500 mL glass graduated cylinder, add 400 mL of purified water and 7.5 mL of acetic acid. Dissolve using a stirrer bar then adjust the pH to 4.2 ± 0.1 , adjust with ammonia or acetic acid (LCMS grade) if necessary. Add additional water to make up to 500 mL, filter the solution through a 0.20 μ m nylon filter.

Storage: 2-8 °C

Expiry: 1 month

Mobile Phase A: 5 mM Ammonium Acetate Buffer Solution, pH 4.2

Transfer 950 mL Milli-Q water and 50 mL of 100 mM ammonium acetate Buffer pH 4.2 to a glass bottle. Mix well.

Storage: Ambient

Expiry: 1 month

Mobile Phase B: 5 mM Ammonium Acetate/Isopropanol/Acetonitrile (5:62:33)

Add 100 mL of 100 mM Ammonium Acetate Buffer pH 4.2, 1240 mL of isopropanol, and 660 mL of acetonitrile to a glass bottle. Mix well.

Storage: Ambient

Expiry: 1 month

Instrument conditions

Table 1: UHPLC conditions

Column	Waters Xselect CSH, C18 column (4.6 mm x 150 mm, 3.5 µm)
Flow Rate	0.9 mL/min
Injection Volume	10 µL
Column Temperature	40 °C
Run Time	30 minutes
Auto sampler Temperature	20 °C
Needle wash	90% Methanol
Purge wash	10% Acetonitrile
Seal wash	10% Acetonitrile
Column flush	100% Acetonitrile

Table 2: CAD Parameters:

Evaporation tube temperature	50 °C
Gas Regulation Mode	Analytical
Power Function	1.10
Filter Constant	3.6
Range	500
Output Offset	0%

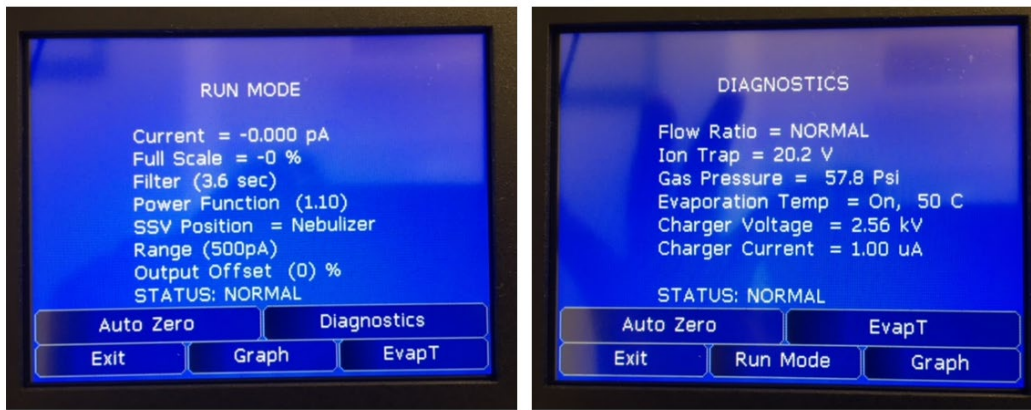


Figure 1: Charged Aerosol Detector (CAD) run mode and diagnostic.

Note: Ensure the CAD parameters are entered correctly (check the entry In both Run mode and Diagnostics LCD display).

Table 3: eSATIN module:

Environment	50 Hz
Sampling rate	2
Scale factor	1000

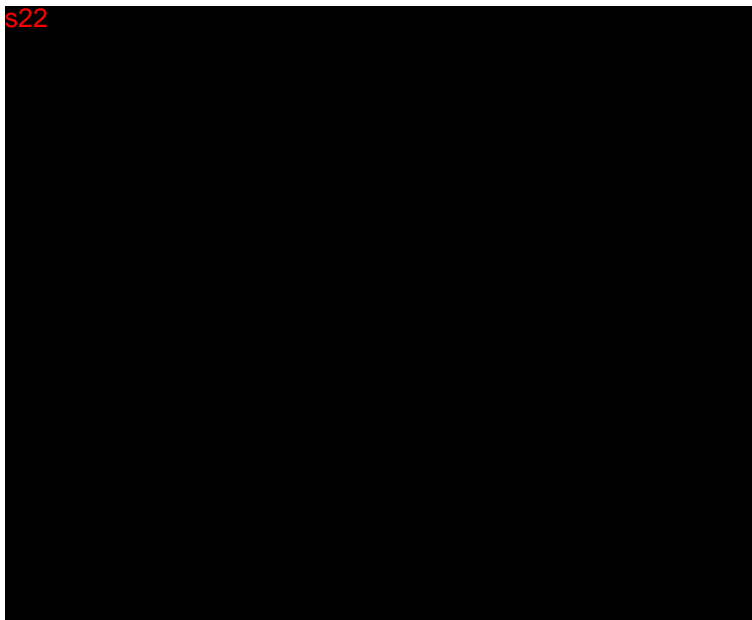


Figure 2: eSATIN module setup

Table 4: Gradient program:

Time (minutes)	%Mobile Phase A	%Mobile Phase B
----------------	-----------------	-----------------

0.0	15	85
2.0	15	85
2.1	11	89
15.0	8	92
19.0	0	100
24.0	0	100
26.0	15	85
30.0	15	85

Standard preparation

Preparation of mixed lipid stock solution (250%)

Accurately weigh each individual lipid according to Table 5 on a micro balance (except for SM102) and transfer into a 50 mL volumetric flask. Pipette SM102 directly into the volumetric flask. Fill the volumetric flask with ethanol up to 90% of the volume, stopper it and sonicate to dissolve. Fill the volumetric flask with ethanol up to the volume and mix well. Store at -20 °C.

When removing from -20 °C storage, the solution is cloudy. Allow the stock solution to reach ambient temperature prior to prepare working standards, if the solution still looks cloudy sonicate to give a clear solution before use.

Table 5: Details for mixed lipid stock solution

Component	Weight (mg)	Final concentration mg/mL
PEG2000-DMG	s47	
Cholesterol		
SM102		
DSPC		

Assay control preparation:

If enough lipid standards are available, prepare a second mixed lipid stock solution as described above. For the assay control dilute 1:2 with ethanol.

Resolution solution preparation:

Prepare separately, 1 mg/mL solution of lyso-PC and 4 mg/mL of stearic acid solution in ethanol. The lyso-PC solution contains lyso-PC isomer.

Add components listed in the Table 6 below and mix well.

Table 6: Stock solution dilutions used to prepare resolution solution

Component	Volume μL
1 mg/mL lyso-PC	25
4 mg/mL stearic acid	25
Mixed lipid stock solution (250%)	400
Ethanol	550

The final resolution standard solution contains: lyso-PC, lyso-PC isomer, PEG 2000 DMG, stearic acid, cholesterol, SM102 and DSPC.

The individual resolution standard component solutions are stable for 6 months at $-20\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$ when stored in a tightly sealed container.

The final diluted resolution standard solution is stable for 6 months at $-20\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$ and for up to 3 days at room temperature in tightly sealed container.

When removing from $-20\text{ }^{\circ}\text{C}$ storage allow standards to equilibrate to room temperature, shake well and then sonicate until a clear, particle-free solution is obtained.

Preparation of mixed lipid Calibration standards

On the day of the analysis, use the mixed lipid stock solution (250%) to prepare the working calibration standards, as described in Table 7. When removing the lipid stock solution from $-20\text{ }^{\circ}\text{C}$ storage, the solution is cloudy. Allow the stock solution to reach ambient temperature prior to prepare working standards, if the solution still looks cloudy sonicate to give a clear solution before use.

Prepare the dilutions with a multipette using ethanol as the diluent. Make the dilution directly into the HPLC vials.

Table 7: Working standard curve preparation

Linearity level	STD Stock sol (μL)	EtOH (μL)	Concentration of total lipid (mg/mL)
Level 6 (250%)	STD stock Solution		s47
Level 5 (200%)	800	200	
Level 4 (150%)	600	400	
Level 3 (100%)	400	600	
Level 2 (50%)	200	800	
Level 1 (25%)	100	900	

Use the amount weighed and purity percentage for the each lipid to determine the actual concentration of the each lipid in calibration standard preparation.

The target (nominal) concentration of the lipids for each level is described in the table below.

Table 8: Lipid concentrations for each calibration level

Linearity level	PEG DMG-2000	Cholesterol	SM-102	DSPC
Level 6 (250%)	S47			
Level 5 (200%)				
Level 4 (150%)				
Level 3 (100%)				
Level 2 (50%)				
Level 1 (25%)				

Preparation of drug product sample solutions

Bring the samples to room temperature gently mix the samples by inverting multiple times prior to dilution.

Note: Do not vortex or sonicate the drug product prior to dilution. This slightly impacts the sample homogeneity and increases particle size of the nano-particles.

Dilute the sample in a 1.5mL Eppendorf by pipetting 250µL drug product and 750 µL ethanol to bring the total lipid concentration close to 1 mg/mL. Two individual preparations should be made for each sample. Centrifuge at 14000 xg for 10 minutes (even though the solution appears clear there will be a pellet at the bottom of the tube), then transfer a sufficient volume of the supernatant to the HPLC vial for analysis. (Be careful not to disturb the pellet at the bottom).

Vortex the HPLC vial gently to mix well.

Sample solutions are stable for up to 3 months at -20 °C

Samples are stable for up to 3 days when stored at 20 °C in the auto sampler. This stability only applies to un-punctured vials.

Instrument setup:

Turn on the UPLC, CAD and eSATIN, place the channel A in mobile phase A and channel B in the mobile phase B.

Purge the system for 10 minutes (not less than 2 minutes for each channel), connect the column and equilibrate the column with initial mobile phase proportions (A- 15% and B- 85%) at 0.9 mL/min until a stable baseline is achieved.

Make one or two injections of the ethanol as blank and make sure no significant peaks are present that could interfere with the integration of the lipids.

Inject the standards and sample following the sequence described in the Table 8 below. Ensure the SST solution is injected in duplicate after every two lots of samples.

Table 8: Example sample set

Sample name	Number of injections
Diluent as Blank	2
Resolution standard	1
Level 3 as SST	3
Level 1	2
Level 2	2
Level 3	2
Level 4	2
Level 5	2
Level 6	2
Assay control/check standard	2
Sample Prep 1	2
Sample Prep 2	2
Diluent as Blank	1
Level 3 as SST	2
Diluent as Blank	1

Data processing and analysis:

Integrate the lipids peak, see figures 4, 5 and 6 for examples of integrated chromatograms.

Using a software program fit the calibration curve using a quadratic equation (if using empower x value is amount and y value is area).

The calibration curve is represented by the equation

$$Y = A + BX + CX^2$$

Where

A = constant term

B = first order coefficient

C = second order coefficient

Y = response of the sample peak calculated by the software

X = component amount

System suitability criteria:

- 1, No significant interference peaks should be observed between 4 and 22 minutes in the last diluent as blank injection.
- 2, The USP resolution between PEG 2000 DMG and stearic acid peaks in the resolution standard injection should be not less than 1.0 (≥ 1.0).
- 3, The RSD of peak areas and retention times of the first three SST injections must be less than or equal to 5.0% ($\leq 5.0\%$).

Assay acceptance criteria:

1. The RSD of peak areas and retention times for the system suitability injections during the run (at least n=5) must be less than or equal to 5.0% ($\leq 5.0\%$).
2. The r^2 of the standard curve must be ≥ 0.995 for all lipids
3. The percentage recovery for the lipids in the assay control preparation must be between 85-115%.
4. % Difference for sample preparations is NMT 5%

$$\text{Calculated as \% Difference samples preparations} = \frac{|\text{Prep1 Conc} \left(\frac{\text{mg}}{\text{mL}}\right) - \text{Prep 2 Conc} \left(\frac{\text{mg}}{\text{mL}}\right)|}{\text{Prep1 Conc} \left(\frac{\text{mg}}{\text{mL}}\right) + \text{Prep2 Conc} \left(\frac{\text{mg}}{\text{mL}}\right)} * 100$$

Lipid identification criteria:

1. The average retention time of the lipids in each sample must be within 5% of the average retention time of the level 3/SST injections. Calculate the % lipid retention time agreement as described below:

$$\% \text{ Lipid Retention time agreement} = \left(\frac{\text{Average RT of lipid sample}}{\left(\text{Average retention time lipid level 3} \frac{\text{standard}}{\text{SST}} \right)} \right) * 100$$

An acceptable value will be from 95-105%.

2. For identity testing, the peak shape(s) of each lipid(s) in the sample must match the peak shape(s) of each lipid(s) in SST injection. Note: retention time of the SM102 can be variable.

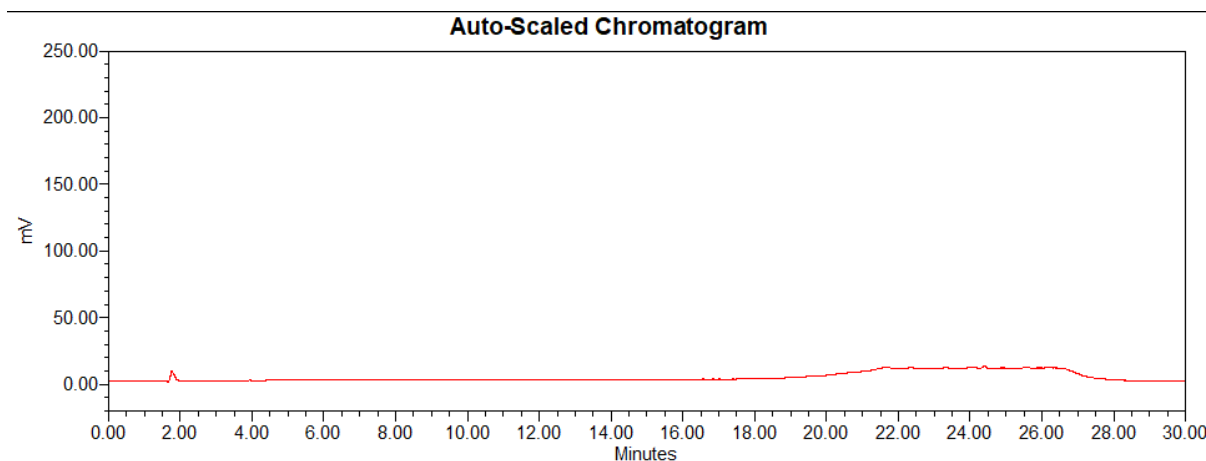
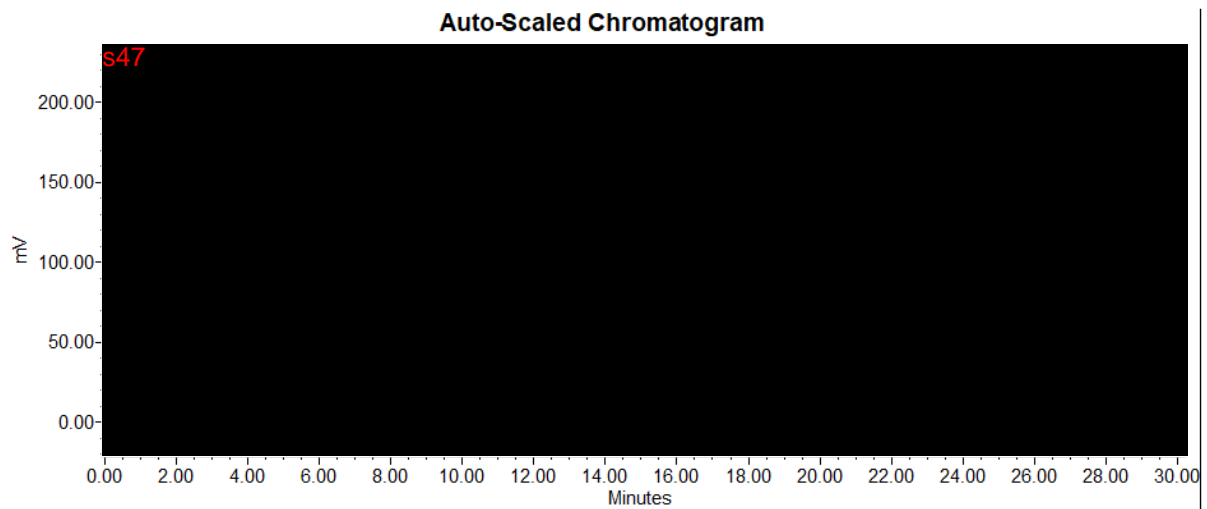
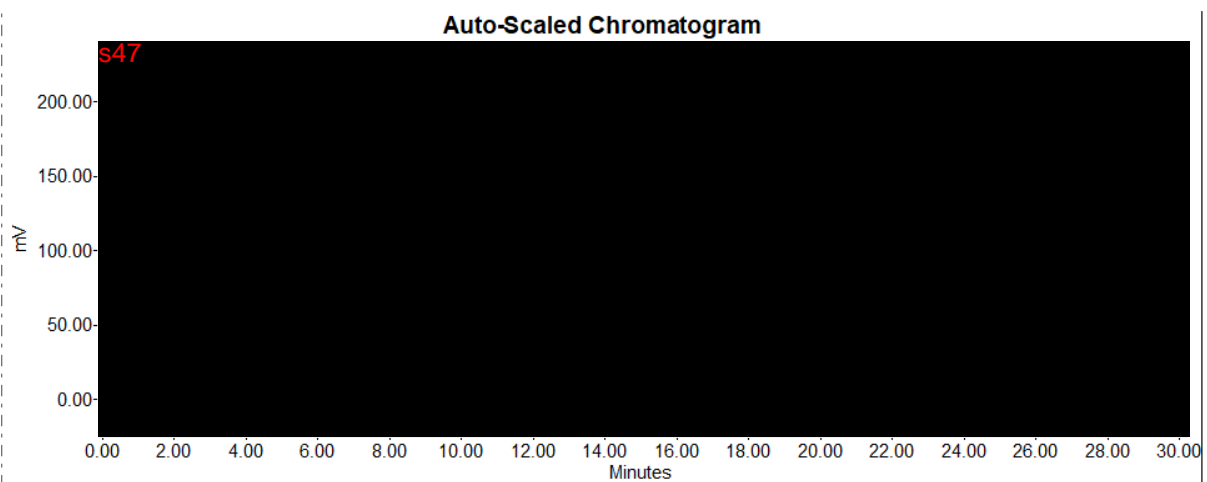
Example chromatograms

Figure 3: Example Blank Chromatogram**Figure 4:** Example Resolution standard solution chromatogram**Figure 5:** Example SST chromatogram

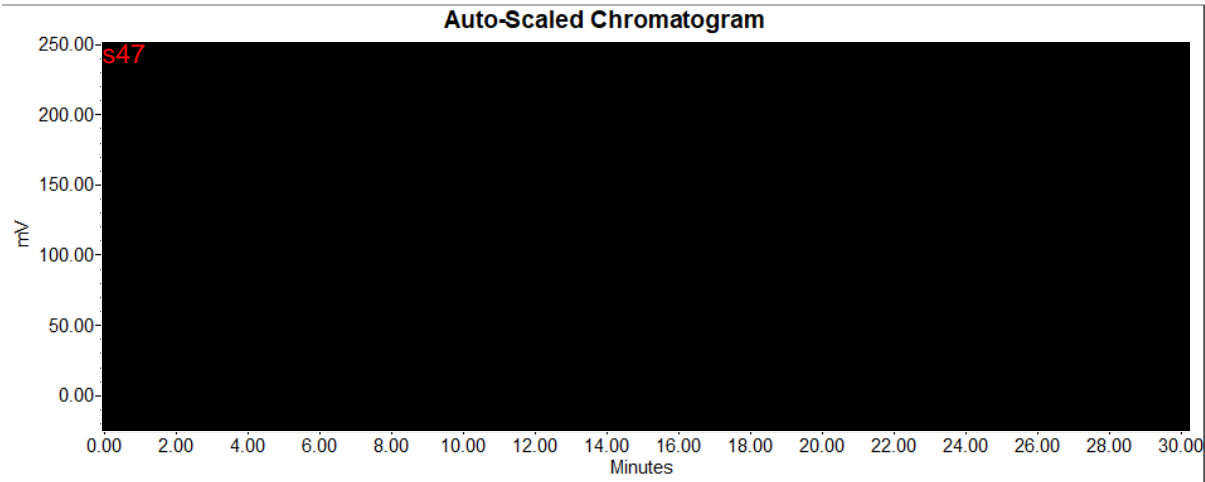


Figure 6: Example Sample chromatogram



Australian Government
 Department of Health and Aged Care
 Therapeutic Goods Administration

Laboratories Branch

Owner: s22 [REDACTED]	Number: Chem-Form-70
Author: s22 [REDACTED]	Version: 1
Active: 22/10/2021	Review: 22/04/2023
Title: Content of Lipids in Moderna vaccine by HPLC CAD	

TGA sample No(s): 2303001127
Lot/Batch number(s): 0000025

SUBSTANCES ASSAYED	PEG 2000 DMG, Cholesterol, SM102 and DSPC.
METHOD REFERENCE: Chem-Method-45	INSTRUMENT No. 20 with CAD detector
Method Modifications approved by:	s22 N/A <input type="checkbox"/>
SYSTEM SUITABILITY REQUIREMENTS MET: Yes	ASSAY REQUIREMENTS MET: Yes

RESULTS:

TGA Sample number(s)	Average lipid content (mg/mL)			
	PEG 2000 DMG	Cholesterol	SM 102	DSPC
s47 [REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
2303001127	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
		s22 09/03/2023		

Identification RT requirements met? (RT ± 5% compared to SST)	Yes
Comments	
Result	Pass
Analyst: s22 [REDACTED]	Date: 9/03/2023
Checked by: s22 [REDACTED]	Date: 9/03/2023

LIPID STANDARDS STOCK SOLUTION PREPARATION DETAILS

Stock solution prepared date:	2/12/2022	Prepared by	s22	Within expiry	Yes
-------------------------------	-----------	-------------	-----	---------------	-----

Note: PEG 2000 DMG individual reference standard used in the Lipid Standard Stock solution expired 02/2023.

Attached <input type="checkbox"/> or Refer to (TRIM Record number/Sample number)	D22-6196482
--	-----------------------------

Concentration of each lipid in the lipid stock solution

	PEG2000DMG	Cholesterol	SM102	DSPC
Concentration in stock solution (mg/mL)	s47			

WORKING STANDARD CURVE PREPARATION DETAILS

Date prepared:	7/03/2023	Prepared by	s22	POVA LIMS	33271
----------------	-----------	-------------	-----	-----------	-------

Level	Mixed Lipid Soln (µL)	EtOH (µL)	DF	Concentration of total lipid content (mg/mL)
1 (25%)	100	900	10	0.25
2 (50%)	200	800	5	0.5
3 (100%)	400	600	2.5	1.0
4 (150%)	600	400	1.66	1.5
5 (200%)	800	200	1.25	2.0
6 (250%)	Standard stock solution		1	2.5

For working standard curve concentration details refer to Lipid standard stock solution preparation record in TRIM.

ASSAY CONTROL STOCK SOLUTION PREPARATION DETAILS

Stock solution prepared date:	2/12/2022	Prepared by	s22	Within expiry	Yes
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Note: PEG 2000 DMG individual reference standard used in the Lipid Standard Stock solution expired 02/2023.

Attached <input type="checkbox"/> or Refer to (TRIM Record number/Sample number)	D22-6196482
--	-----------------------------

Concentration of each lipid in the assay control stock solution

	PEG2000DMG	Cholesterol	SM102	DSPC
Concentration in stock solution (mg/mL)	s47			

WORKING STANDARD ASSAY CONTROL PREPARATION DETAILS

Date prepared:	7/03/2023	Prepared by	s22	POVA LIMS	33271
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Mixed Lipid Soln (µL)	EtOH (µL)	DF	Final Target conc (mg/mL)	Actual conc (mg/mL)
500	500	2	PEG2000DMG	s47
			Cholesterol	
			SM102	
			DSPC	
250	750	4	PEG2000DMG	s47
			Cholesterol	
			SM102	
			DSPC	

EtOH:	Supplier	Supelco	Lot No	I1162683131
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SAMPLE PREPARATION DETAILS

Date prepared	07/03/2023	Prepared by	s22
POVA LIMS	33271	Centrifuge LIMS	33375

Sample test solutions prepared in duplicate as described in Chem-Method-45

Yes or No - refer to method modification section

EtOH:	Supplier	Supelco	Lot No	11162683131
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ATTACHMENTS:

System suitability report, Calibration plots, Result summary report, Example chromatograms, Run and method summary and mobile phase preparation details (attached or refer to TRIM record/sample number [D23-5194968](#))

<p>Method Modifications</p> <p>Injection volume was increased to 20 µL for samples and the assay control 1:3 to accommodate for the type of sample.</p>										
<p>Comments</p> <p>Resolution standard solution preparation: Refer to D22-5016245 for preparation of 1 mg/mL lyso-PC and 4 mg/mL stearic acid solutions.</p> <p>Added components listed in the table below and mixed well.</p> <p>Each component in the resolution standard solution</p> <table border="1"> <thead> <tr> <th>Component</th> <th>Volume µL</th> </tr> </thead> <tbody> <tr> <td>1 mg/mL lyso-PC</td> <td>25</td> </tr> <tr> <td>4 mg/mL stearic acid</td> <td>25</td> </tr> <tr> <td>Mixed lipid stock solution (250%)</td> <td>400</td> </tr> <tr> <td>Ethanol</td> <td>550</td> </tr> </tbody> </table> <p>The final resolution standard solution contains: lyso-PC, lyso-PC isomer, PEG 2000 DMG, stearic acid, cholesterol, SM102 and DSPC.</p> <p>Mixed lipid stock solution was prepared on 02 Dec 2022 by s22 Working resolution standard solution prepared on 07 March 2023 by s22</p>	Component	Volume µL	1 mg/mL lyso-PC	25	4 mg/mL stearic acid	25	Mixed lipid stock solution (250%)	400	Ethanol	550
Component	Volume µL									
1 mg/mL lyso-PC	25									
4 mg/mL stearic acid	25									
Mixed lipid stock solution (250%)	400									
Ethanol	550									



Owner: s22	Number: Chem-Method-45
Author: s22	Version: 2
Active: 3/12/2021	Review: 29/03/2024
Title: Identification and Quantitation of PEG 2000 DMG, Cholesterol, SM-102 and DSPC in Moderna Vaccine	

Purpose

The purpose of this method is the identification and quantitation for the found lipids that are part of the COVID-19 vaccine Spikevax. These include: PEG 2000 DMG, cholesterol, SM-102 and DSPC.

Scope

This method applies to drug product (DP) samples with a nominal concentration s47 mRNA tested in Laboratories Branch at TGA.

Abbreviations

µL	Microlitre
CAD	Charged Aerosol Detection
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
LCMS	Liquid Chromatography Mass Spectrometry
Lyso-PC	1-Stearoyl-sn-glycerol-3-phosphatidylcholine
PEG 2000 DMG	1,2-Dimyristoyl-sn-glycero-3-methoxypolyethylene glycol
RP-UHPLC	Reverse Phase Ultra-High Performance Liquid Chromatography
RT	Retention Time
SM102	Heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate
SST	System suitability
STD	Standard

Method Reference

The method is based on the method for Identification and Quantitation of Lipids in s22 and determination of lipid content, purity and identity by UPLCCAD for SM102/PEG formulations from Moderna.

Equipment, Materials and Reagents

Equipment
Waters Acquity UPLC H-Class equipped with QSM to deliver gradient flow at 0.9 mL/min, SM-FTN, column oven, charged aerosol detector and eSAT/IN.
Waters Xselect CSH, C18 column, 4.6 mm x 150 mm, 3.5 μ m (Waters part No. 186005270)
VanGuard Cartridge Holder (Waters part No. 186007949)
XSelect CSH VanGuard Cartridge, 130 A°, 3.5 μ m x 5 mm (Waters part No. 186007813)
Analytical Balance, capable of reading to 0.1 mg
Centrifuge, capable of 14,000 x g (eg LIMS 32473 (Biotherapeutics))
Sonication Bath
Eppendorf multipette
Vortex
Volumetric flasks
pH meter

Materials
Agilent Glass screw top, high recovery, HPLC vials (part No. 5183-2030) with Waters LCMS certified caps and pre-slit PTFE/Silicone septum (part No. 186005827).
Suitable combitips, recommend 1mL

Reagents
Ultra pure water, resistivity needs to be => 18.2 M Ω (LIMS 33031 is recommended)
Isopropanol (IPA), LCMS Grade
Methanol (MeOH), LCMS Grade
Acetonitrile (ACN), HPLC Grade
Ethanol absolute for analysis (EtOH), ACS Reagent
Ammonium acetate, LCMS Grade
Acetic acid, LCMS Grade
Ammonia solution, LCMS Grade

Mobile phase preparation

100 mM ammonium acetate buffer pH 4.2

Weigh 3.85 g of ammonium acetate into a weight boat, transfer to a 500 mL glass graduated cylinder, add 400 mL of purified water and 7.5 mL of acetic acid. Dissolve using a stirrer bar then adjust the pH to 4.2 \pm 0.1, adjust with ammonia or acetic acid (LCMS grade) if necessary. Add additional water to make up to 500 mL, filter the solution through a 0.20 μ m nylon filter.

Storage: 2-8 °C

Expiry: 1 month

Mobile Phase A: 5 mM Ammonium Acetate Buffer Solution, pH 4.2

Transfer 950 mL Milli-Q water and 50 mL of 100 mM ammonium acetate Buffer pH 4.2 to a glass bottle. Mix well.

Storage: Ambient

Expiry: 1 month

Mobile Phase B: 5 mM Ammonium Acetate/Isopropanol/Acetonitrile (5:62:33)

Add 100 mL of 100 mM Ammonium Acetate Buffer pH 4.2, 1240 mL of isopropanol, and 660 mL of acetonitrile to a glass bottle. Mix well.

Storage: Ambient

Expiry: 1 month

Instrument conditions**Table 1:** UHPLC conditions

Column	Waters Xselect CSH, C18 column (4.6 mm x 150 mm, 3.5 µm)
Flow Rate	0.9 mL/min
Injection Volume	10 µL
Column Temperature	40 °C
Run Time	30 minutes
Auto sampler Temperature	20 °C
Needle wash	90% Methanol
Purge wash	10% Acetonitrile
Seal wash	10% Acetonitrile
Column flush	100% Aceonitrile

Table 2: CAD Parameters:

Evaporation tube temperature	50 °C
Gas Regulation Mode	Analytical
Power Function	1.10
Filter Constant	3.6
Range	500
Output Offset	0%

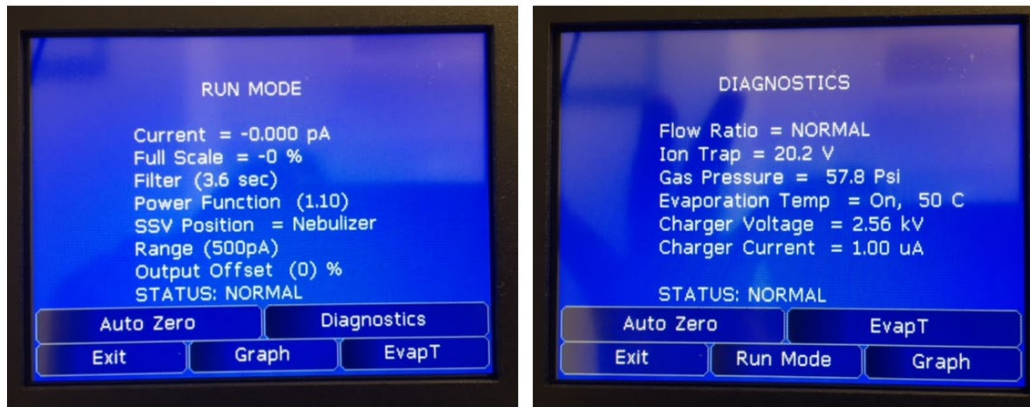


Figure 1: Charged Aerosol Detector (CAD) run mode and diagnostic.

Note: Ensure the CAD parameters are entered correctly (check the entry In both Run mode and Diagnostics LCD display).

Table 3: eSATIN module:

Environment	50 Hz
Sampling rate	2
Scale factor	1000

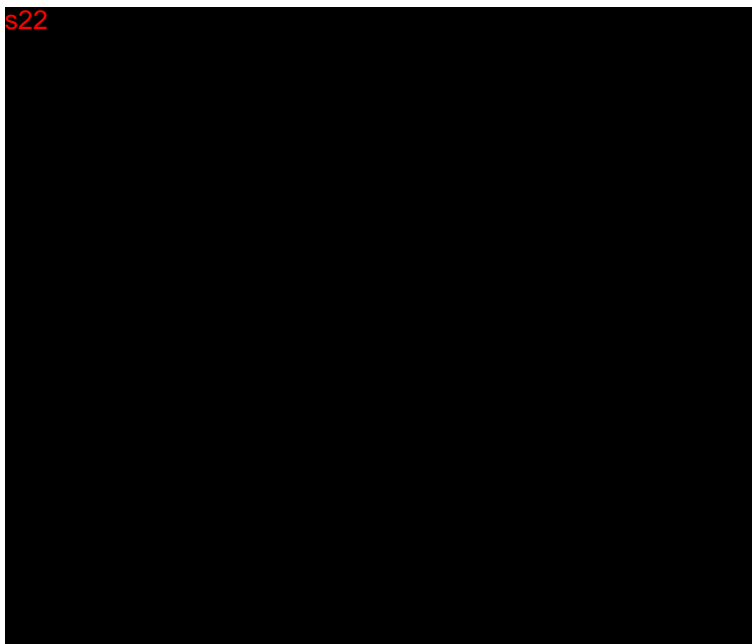


Figure 2: eSATIN module setup

Table 4: Gradient program:

Time (minutes)	%Mobile Phase A	%Mobile Phase B
0.0	15	85
2.0	15	85
2.1	11	89
15.0	8	92
19.0	0	100
24.0	0	100
26.0	15	85
30.0	15	85

Standard preparation

Preparation of mixed lipid stock solution (250%)

Accurately weigh each individual lipid according to Table 5 on a micro balance (except for SM102) and transfer into a 50 mL volumetric flask. Pipette SM102 directly into the volumetric flask. Fill the volumetric flask with ethanol up to 90% of the volume, stopper it and sonicate to dissolve. Fill the volumetric flask with ethanol up to the volume and mix well. Store at -20 °C.

When removing from -20 °C storage, the solution is cloudy. Allow the stock solution to reach ambient temperature prior to prepare working standards, if the solution still looks cloudy sonicate to give a clear solution before use.

Table 5: Details for mixed lipid stock solution

Component	Weight (mg)	Final concentration mg/mL
PEG2000-DMG	s47	
Cholesterol		
SM102		
DSPC		

Assay control preparation:

If enough lipid standards are available, prepare a second mixed lipid stock solution as described above. For the assay control dilute 1:1 with ethanol.

Resolution solution preparation:

Prepare separately, 1 mg/mL solution of lyso-PC and 4 mg/mL of stearic acid solution in ethanol. The lyso-PC solution contains lyso-PC isomer.

Add components listed in the Table 6 below and mix well.

Table 6: Stock solution dilutions used to prepare resolution solution

Component	Volume μL
1 mg/mL lyso-PC	25
4 mg/mL stearic acid	25
Mixed lipid stock solution (250%)	400
Ethanol	550

The final resolution standard solution contains: lyso-PC, lyso-PC isomer, PEG 2000 DMG, stearic acid, cholesterol, SM102 and DSPC.

The individual resolution standard component solutions are stable for 6 months at $-20\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$ when stored in a tightly sealed container.

The final diluted resolution standard solution is stable for 6 months at $-20\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$ and for up to 3 days at room temperature in tightly sealed container.

When removing from $-20\text{ }^{\circ}\text{C}$ storage allow standards to equilibrate to room temperature, shake well and then sonicate until a clear, particle-free solution is obtained.

Preparation of mixed lipid Calibration standards

On the day of the analysis, use the mixed lipid stock solution (250%) to prepare the working calibration standards, as described in Table 7. When removing the lipid stock solution from $-20\text{ }^{\circ}\text{C}$ storage, the solution is cloudy. Allow the stock solution to reach ambient temperature prior to prepare working standards, if the solution still looks cloudy sonicate to give a clear solution before use.

Prepare the dilutions with a multipette using ethanol as the diluent. Make the dilution directly into the HPLC vials.

Table 7: Working standard curve preparation

Linearity level	STD Stock sol (µL)	EtOH (µL)	Concentration of total lipid (mg/mL)
Level 6 (250%)	STD stock Solution		s47
Level 5 (200%)	800	200	
Level 4 (150%)	600	400	
Level 3 (100%)	400	600	
Level 2 (50%)	200	800	
Level 1 (25%)	100	900	

Use the amount weighed and purity percentage for the each lipid to determine the actual concentration of the each lipid in calibration standard preparation.

The target (nominal) concentration of the lipids for each level is described in the table below.

Table 8: Lipid concentrations for each calibration level

Linearity level	PEG DMG-2000	Cholesterol	SM-102	DSPC
Level 6 (250%)	s47			
Level 5 (200%)				
Level 4 (150%)				
Level 3 (100%)				
Level 2 (50%)				
Level 1 (25%)				

Preparation of drug product sample solutions

Bring the samples to room temperature gently mix the samples by inverting multiple times prior to dilution.

Note: Do not vortex or sonicate the drug product prior to dilution. This slightly impacts the sample homogeneity and increases particle size of the nano-particles.

Dilute the sample in a 1.5mL Eppendorf by pipetting 250µL drug product and 750 µL ethanol to bring the total lipid concentration close to 1 mg/mL. Two individual preparations should be made for each sample. Centrifuge at 14000 xg for 10 minutes (even though the solution appears clear there will be a pellet at the bottom of the tube), then transfer a sufficient volume of the supernatant to the HPLC vial for analysis. (Be careful not to disturb the pellet at the bottom).

Vortex the HPLC vial gently to mix well.

Sample solutions are stable for up to 3 months at -20 °C

Samples are stable for up to 3 days when stored at 20 °C in the auto sampler. This stability only applies to un-punctured vials.

Instrument setup:

Turn on the UPLC, CAD and eSATIN, place the channel A in mobile phase A and channel B in the mobile phase B.

Purge the system for 10 minutes (not less than 2 minutes for each channel), connect the column and equilibrate the column with initial mobile phase proportions (A- 15% and B- 85%) at 0.9 mL/min until a stable baseline is achieved.

Make one or two injections of the ethanol as blank and make sure no significant peaks are present that could interfere with the integration of the lipids.

Inject the standards and sample following the sequence described in the Table 8 below. Ensure the SST solution is injected in duplicate after every two lots of samples.

Table 8: Example sample set

Sample name	Number of injections
Diluent as Blank	2
Resolution standard	1
Level 3 as SST	3
Level 1	2
Level 2	2
Level 3	2
Level 4	2
Level 5	2
Level 6	2
Assay control/check standard	2
Sample Prep 1	2
Sample Prep 2	2
Diluent as Blank	1
Level 3 as SST	2
Diluent as Blank	1

Data processing and analysis:

Integrate the lipids peak, see figures 4, 5 and 6 for examples of integrated chromatograms.

Using a software program fit the calibration curve using a quadratic equation (if using empower x value is amount and y value is area).

The calibration curve is represented by the equation

$$Y = A + BX + CX^2$$

Where

A = constant term

B = first order coefficient

C = second order coefficient

Y = response of the sample peak calculated by the software

X = component amount

System suitability criteria:

- 1, No significant interference peaks should be observed between 4 and 22 minutes in the last diluent as blank injection.
- 2, The USP resolution between PEG 2000 DMG and stearic acid peaks in the resolution standard injection should be not less than 1.0 (≥ 1.0).
- 3, The RSD of peak areas and retention times of the first three SST injections must be less than or equal to 5.0% ($\leq 5.0\%$).

Assay acceptance criteria:

1. The RSD of peak areas and retention times for the system suitability injections during the run (at least n=5) must be less than or equal to 5.0% ($\leq 5.0\%$).
2. The r^2 of the standard curve must be ≥ 0.995 for all lipids
3. The percentage recovery for the lipids in the assay control preparation must be between 85-115%.
4. % Difference for sample preparations is NMT 5%

$$\text{Calculated as \% Difference samples preparations} = \frac{\left| \text{Prep1 Conc} \left(\frac{\text{mg}}{\text{mL}} \right) - \text{Prep 2 Conc} \left(\frac{\text{mg}}{\text{mL}} \right) \right|}{\text{Prep1 Conc} \left(\frac{\text{mg}}{\text{mL}} \right) + \text{Prep2 Conc} \left(\frac{\text{mg}}{\text{mL}} \right)} * 100$$

Lipid identification criteria:

1. The average retention time of the lipids in each sample must be within 5% of the average retention time of the level 3/SST injections. Calculate the % lipid retention time agreement as described below:

$$\% \text{ Lipid Retention time agreement} = \left(\frac{\text{Average RT of lipid sample}}{\left(\text{Average retention time lipid level 3} \frac{\text{standard}}{\text{SST}} \right)} \right) * 100$$

An acceptable value will be from 95-105%.

2. For identity testing, the peak shape(s) of each lipid(s) in the sample must match the peak shape(s) of each lipid(s) in SST injection. Note: retention time of the SM102 can be variable.

Example chromatograms

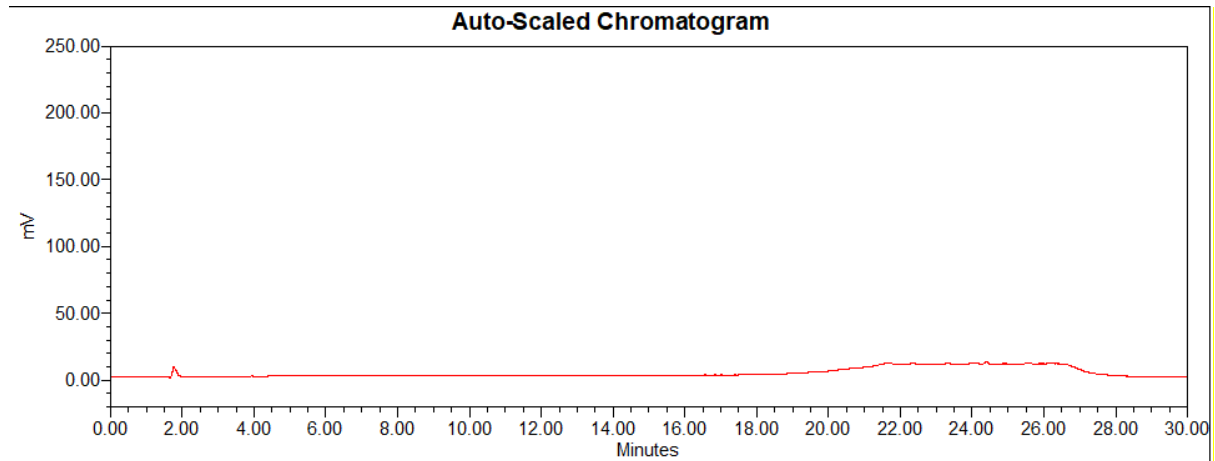


Figure 3: Example Blank Chromatogram

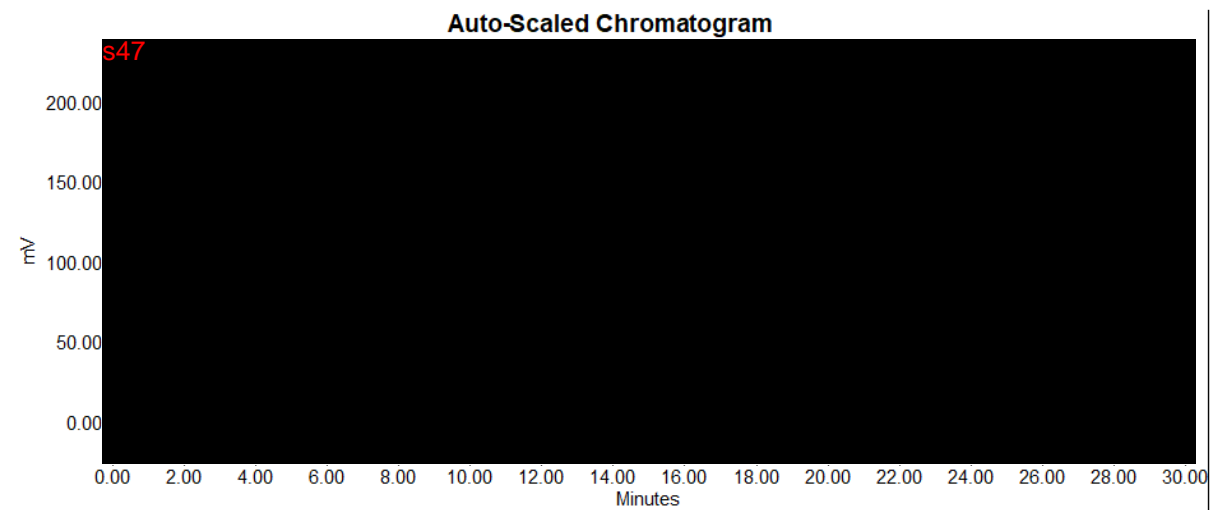


Figure 4: Example Resolution standard solution chromatogram

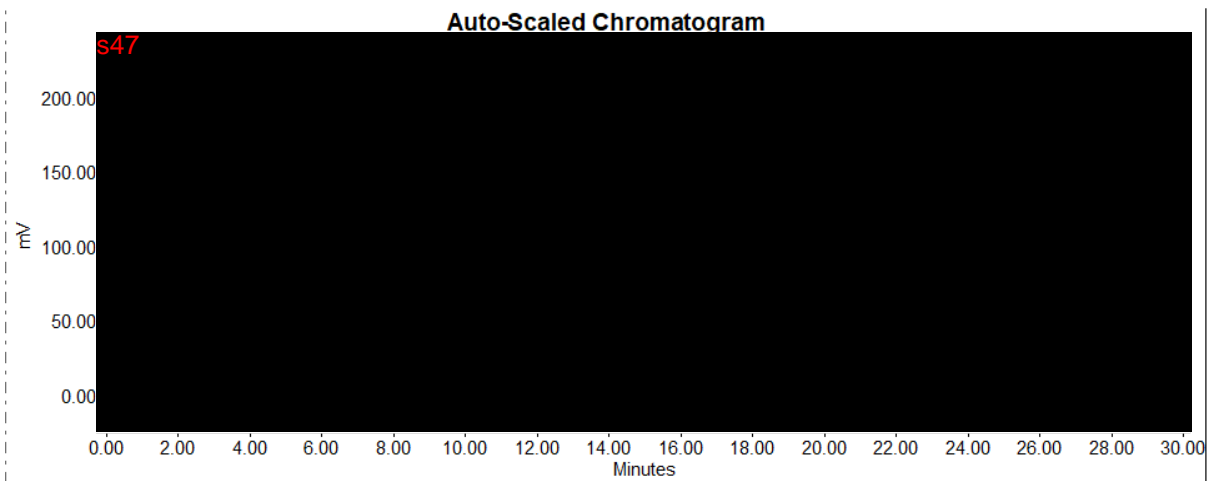


Figure 5: Example SST chromatogram

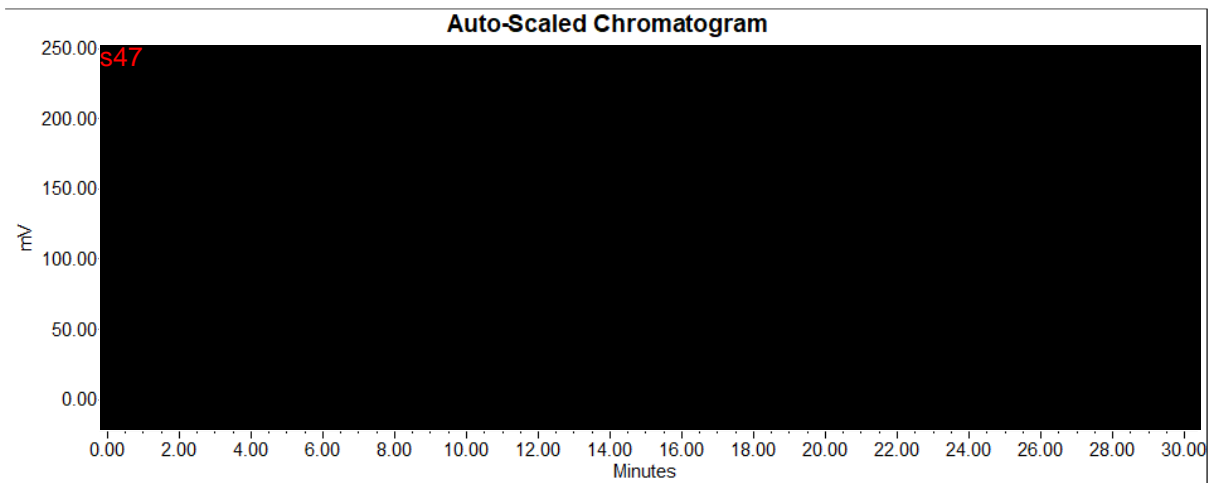


Figure 6: Example Sample chromatogram



Australian Government

Department of Health and Aged Care

Therapeutic Goods Administration

Nonclinical Evaluation Report

Elasomeran/davesomeran [SPIKEVAX BIVALENT ORIGINAL/OMICRON BA.4-5]

Submission No: PM-2022-04824-1-2

Sponsor: Moderna Australia Pty Ltd

16 January 2023 (Round 1)

25 January 2023 (Round 2)

TGA Health Safety
Regulation

NONCLINICAL EVALUATION REPORT**Submission type:** New vaccine**Sponsor:** Moderna Australia Pty Ltd**Generic name:** Elasomeran/davesomeran**Trade name:** SPIKEVAX BIVALENT ORIGINAL/OMICRON BA.4-5
COVID-19 VACCINE**Dose form and strength:** Injection, Suspension; 100 µg in 1 mL**Vaccine Type:** mRNA encapsulated in LNP**Submission No:** PM-2022-04824-1-2**Tox file No:** E22-627993**TRIM reference:** D23-5004129**Date authorised:** 16 January 2023 (Round 1)

25 January 2023 (Round 2)

**This report has been revised to incorporate the sponsor's s.31 response
and replaces the Nonclinical Evaluation Report issued at Round 1**

Note: This evaluation report has been peer-reviewed and is authorised for release to the sponsor.

TABLE OF CONTENTS

SUMMARY, ASSESSMENT AND RECOMMENDATION	4
APPENDIX 1 EVALUATION OF NONCLINICAL DATA	8
PRIMARY PHARMACODYNAMICS	8
1.1. BA.4/BA.5 SPIKE PROTEIN EXPRESSION <i>IN VITRO</i>	8
1.2. IMMUNOGENICITY IN MICE	10
1.3. IMMUNOGENICITY AND PROTECTION	13

SUMMARY, ASSESSMENT AND RECOMMENDATION

- Moderna Australia Pty Ltd has applied for provisional approval of a new COVID-19 vaccine (SPIKEVAX BIVALENT ORIGINAL/OMICRON BA.4-5; mRNA-1273.222), containing elasomeran and davesomeran in lipid nanoparticles (LNP). The ORIGINAL/OMICRON BA.4-5 bivalent vaccine is indicated as a booster dose for active immunisation to prevent coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 in individuals 6 years of age and older who previously received at least a primary vaccination course against COVID-19.
- The vaccine dose in adults is 50 µg of mRNA/0.5 mL (25 µg of each mRNA: elasomeran [mRNA-1273] and davesomeran [mRNA-1273.045]), and in children 6 years to <12 years of age the dose is 25 µg of mRNA/0.25 mL (12.5 µg of each mRNA) given intramuscularly (IM) at least 3 months following a primary series and/or previous booster dose with SPIKEVAX (original), SPIKEVAX BIVALENT ORIGINAL/OMICRON or another authorised/approved COVID-19 vaccine, in accordance with official recommendations.
- The new bivalent vaccine (ORIGINAL/OMICRON BA.4-5) is manufactured using the same mRNA platform and manufacturing method as the provisionally approved Original/Omicron BA.1 bivalent vaccine.
- Module 4 comprised of 3 pharmacology studies:
 - *In vitro* expression of BA.4/BA.5 mRNA in the BIVALENT ORIGINAL/OMICRON BA.4-5 vaccine.
 - Evaluation of immunogenicity of the BIVALENT ORIGINAL/OMICRON BA.4-5 vaccine as well as Omicron-matched vaccines in mice (primary series).
 - Evaluation of protection and immunogenicity from a booster dose of BIVALENT ORIGINAL/OMICRON BA.4-5 vaccine after primary series vaccination with the original monovalent vaccine in mice.
- In the mouse studies, the bivalent Original/Omicron BA.1 (mRNA-1273.214) and monovalent vaccines, mRNA-1273 (original), mRNA-1273.045 (BA.4-5 monovalent) and/or mRNA-1273.529 (BA.1 monovalent) were included for comparison. The following summary and discussion focus on the new bivalent vaccine mRNA-1273.222.
- Expression of SARS-CoV-2 S-2P *in vitro* was evident in Expi293 cells transfected with mRNA-1273.222 based on binding to the N-terminal domain (NTD) of the spike protein (wild type and variants) and hACE-2.
- A 2-dose primary series vaccination with the bivalent mRNA-1273.222 vaccine in mice induced high IgG to the wild type, BA.1 and BA.4-5 spike proteins. As expected, the IgG titres were markedly increased after the 2nd dose cf. the titres after the 1st dose. The bivalent mRNA-1273.222 vaccine elicited high neutralising antibodies (nAb) against BA.4/BA.5 based on pseudovirus (vesicular stomatitis virus and lentivirus) assays. However, the bivalent vaccine induced lower nAb against the Wuhan strain (WA1/D614G) than mRNA-1273, possibly related to the lower mRNA dose (0.5 µg mRNA-1273 in the bivalent vaccine cf. 1 µg mRNA-1273 in the mRNA-1273 monovalent vaccine). The mRNA-1273.045 BA.4-5 monovalent vaccine induced nAb against BA.4-5 similar to that by mRNA-1273.222.

- A booster dose of mRNA-1273.222 after primary series vaccination with mRNA-1273 in K18-hACE2 transgenic mice induced nAb against BA.5, BA.1, Delta and WA1 by the focus reduction neutralisation test using authentic virus. The nAb against BA.5 were lower than that against WA1 and Delta (EC_{50} 276 *cf.* 3040 and 1817, respectively). In comparison, the monovalent mRNA-1273 vaccine elicited no or very low nAb against BA.5 or BA.1. Thus, the mRNA-1273.222 bivalent vaccine as a booster dose after two primary series of the original vaccine induced greater cross-variant neutralisation than the mRNA-1273 monovalent vaccine. Interestingly, the mRNA-1273.214 bivalent vaccine induced nAb against BA.5 similar to that by mRNA-1273.222 (EC_{50} 298 *cf.* 276), but slightly higher nAb against BA.1 (EC_{50} 413 *cf.* 190) than by mRNA-1273.222.
- mRNA-1273.222, as well as mRNA-1273.214 and mRNA-1273, significantly reduced lung, nasal wash and nasal turbinate viral load (measured as RNA or infectious virus) in mice challenged with Omicron BA.5, and reduction in lung viral load was greater by mRNA-1273.222 than by mRNA-1273, but similar to that by mRNA-1273.214.
- Both bivalent vaccines, mRNA-1273.222 and mRNA-1273.214, as a booster dose protected the mice from the development of lung pathology (*cf.* focal pathology in mice boosted with mRNA-1273), although control mice challenged with Omicron BA.5 developed milder lung pathology than that reported with other Omicron subvariants or other SARS-CoV-2 strains. The bivalent vaccines also reduced inflammatory cytokines and chemokines in the lung, compared with mRNA-1273.
- Overall, boosting with either mRNA-1273.222 or mRNA-1273.214 enhanced protection against BA.5 infection compared with protection by boosting with mRNA-1273.
- No toxicity studies on the bivalent vaccine were submitted. This is acceptable since the new mRNA (davesomeran) uses the same backbone and manufacture platform as elasomeran and there are no changes to vaccine formulation except for the additional mRNA.
- There are no nonclinical objections to the provisional approval of the BIVALENT ORIGINAL/OMICRON BA.4-5 vaccine.
- A minor change to the draft Product Information (v1.2 07 Dec22) is recommended:

4.6 FERTILITY, PREGNANCY AND LACTATION

Effects on fertility

“Animal studies with SPIKEVAX do not indicate direct or indirect harmful effects with respect to reproductive toxicity in females.

In a combined fertility and developmental toxicity study, 100 micrograms of mRNA (elasomeran) and other ingredients included in a single human dose of SPIKEVAX (original) was administered to female rats by the intramuscular route on four occasions: 28 and 14 days prior to mating, and on gestation days 1 and 13. SARS-CoV-2 antibody responses were present in dams from prior to mating to the end of the study on lactation day 21 as well as in fetuses and offspring. There were no vaccine related adverse effects on female fertility, pregnancy, embryofetal or offspring development or postnatal development. No data are available on SPIKEVAX (original), SPIKEVAX BIVALENT ORIGINAL/OMICRON (BA.1) or SPIKEVAX BIVALENT ORIGINAL/OMICRON BA.4-5 vaccine placental transfer or excretion in milk. The effect on male fertility has not been determined.”

- Comments on the Sponsor's Responses to Questions raised in the ROUND 1 evaluation:

1. In Study MOD-045EXP, mAb CC40.8, which binds to the NTD of the original Wuhan-Hu-1 and variants spike protein, was used to detect spike protein expression. In Figure 1, panel B, mAb CC40.8 detected spike protein expression following transfection with mRNA 1273.222, but not following transfection with mRNA-1283, which encodes the NTD and RBD of the Wuhan-Hu-1 spike protein. Why did mAb CC40.8 detect NTD of the spike protein expressed by mRNA 1273.222, but not NTD expressed by mRNA-1283? Please explain.

Sponsor's response:

A mistake was made in the description of the binding site for the CC40.8 mAb. It is stated the text and in the legend of the figure the following:

"CC40.8 mAb is specific for the NTD of both the original Wuhan-Hu-1 spike as well as all variants that have emerged to date; in Figure 1B above, CC40.8 binds to a conserved region on the S2 subdomain."

The correct binding site for the CC40.8 mAb is the S2 subdomain, in a site that is conserved between the original Wuhan-hu-1 and all known variants to date. As the S2 subdomain is not present on the mRNA-1283 design antigen, no binding was expected nor measured.

Comment on the sponsor's response:

The sponsor's response is acceptable. The sponsor should provide an updated study report with correct information. The main body of this evaluation report [Appendix 1] has not been updated, and will be corrected upon receipt of the sponsor's updated study report.

2. Study WASHU-K18-89 appears to be published by Scheaffer *et al.* (2022). However, the serum neutralisation EC₅₀ values in the submitted study report (Figure 1) are different from the results in the published article (Extended data Fig. 5). Please explain.

Sponsor's response:

The serum neutralisation analysis provided in WASHU-K18-89 used an LOD of 1:60 dilution. Subsequently, in preparation of the results for publication, a lower LOD of 1:30 was used in a re-analysis of some of the samples, which changed the EC₅₀ values and the LOD in the published manuscript.

Comment on the sponsor's response:

The sponsor's response is acceptable. The main body of this report [Appendix 1] has been updated with these details in addition to depicting the results of the serum neutralisation analysis in mice from the published article. However, it is noted in the published study that the serum neutralisation analysis was a 1:60 dilution (not 1:30 as indicated above) in the assays against the wild type (WT) and Delta B.1.617.2 variant. Noting the same dilution, the results in the submitted study report are still different from the results in the published article. The sponsor is requested to explain.

3. The sponsor is also requested to provide the original lung pathology and cytokine/chemokine results for Study WASHU-K18-89.

Sponsor's response:

Moderna had not planned to update study report WASHU-K18-89 with the lung pathology and cytokine/chemokine results given the publication of these results in *Nature Medicine* manuscript: However, an updated study report can be prepared for submission by the end of Q1 (31 March 2023). As such, the sponsor proposes that the submission of the updated report WASHU-K18-89 including the lung pathology and cytokine/chemokine

results be considered a post approval commitment.

Comment on the sponsor's response:

The sponsor's response is acceptable. Submission of the revised report post provisional approval is acceptable.

- Questions for the Sponsor (ROUND 2):

1. Please provide an updated report for Study MOD-045EXP with corrected statements for the binding site for the CC40.8 mAb. The updated report may be provided post provisional approval.
2. Please provide an updated study report with the original lung pathology and cytokine/chemokine results for Study WASHU-K18-89. The updated report may be provided post provisional approval.
3. The serum neutralisation analysis results for the wild type (WT) and Delta B.1.617.2 variant in the published article (Scheaffer *et al.* 2022) were from assays with an LOD of 1:60 dilution (not 1:30 as indicated by the sponsor), which was the same dilution (1:60) for the study results documented in the submitted study report (WASHU-K18-89). The sponsor is requested to explain why the results in the submitted study report are still different from the results in the published article.

APPENDIX 1 EVALUATION OF NONCLINICAL DATA

New *in vitro* (1) and animal (2) studies were submitted in Module 4 supporting the provisional approval of the new bivalent COVID-19 vaccine in lipid nanoparticles (LNP), containing elasomeran/davesomeran (SPIKEVAX BIVALENT ORIGINAL/OMICRON BA.4-5).

These have been assessed and reported below.

Laboratory codes: **mRNA-1273** = Original Wuhan
mRNA-1273.045 = Omicron BA.4-5
mRNA-1273.222 = Original + Omicron BA.4-5 bivalent
mRNA-1283 = NTD and RBD of the spike protein Wuhan strain
mRNA-1273.214 = Original + BA.1 bivalent
mRNA-1273.529 = = Omicron BA.1

PRIMARY PHARMACODYNAMICS

1.1. BA.4/BA.5 SPIKE PROTEIN EXPRESSION *IN VITRO*

Table 1. Summary of *in vitro* study

Study details & Major findings	
<i>In vitro</i> expression of BA.4/BA.5 mRNA (component of the BIVALENT ORIGINAL/OMICRON BA.4-5 vaccine) Study MOD-045EXP	
Study Design	<ul style="list-style-type: none"> • The objective was to evaluate the <i>in vitro</i> spike protein expression by flow cytometry. Test articles were: <ul style="list-style-type: none"> ○ (1) mRNA in the monovalent mRNA-1273.045 (davesomeran) vaccine that encodes SARS-CoV-2 S-2P antigen of the BA.4/BA5 subvariants of Omicron. ○ (2) mRNA contained in the monovalent mRNA-1283 vaccine encoding the N-terminal domain [NTD] and receptor-binding domain [RBD] of the Wuhan-Hu-1 spike protein linked together and inserted into the cell membrane with a transmembrane domain [NTD-RBD-HATM [<i>positive control</i>]]. • Expression was measured in Expi293 cells after transfection with mRNA. Three detection reagents were: <ul style="list-style-type: none"> ○ (1) mAb CC40.8, specific for NTD of original Wuhan-Hu-1 spike as well as variants ○ (2) mAb CR3022 this antibody selectively binds to the original Wuhan-Hu-1 spike protein in the RBD ○ (3) a recombinant hACE-2, used to measure expression as hACE-2 which binds to the receptor binding motif [RBM] in the original Wuhan-Hu-1 spike as well as all variants. ○ The mRNA contained in mRNA-1283 was used as a positive control as it is known to bind to the CR3022 mAb.
Results & Conclusion	
<ul style="list-style-type: none"> • Evaluation of BA.4/BA.5 mRNA (mRNA-1273.045; davesomeran) at 100 and 500 ng/mL demonstrated expression of SARS-CoV-2 S-2P (based on frequency and mean fluorescence intensity [MFI] of cell surface expression) in Expi293 cells over 48 hours (see Figure 1 below)- there was an overall increase in the frequency of cells expressing the BA.4/BA.5 SARS-CoV-2 S-2P, evident with CC40.8 mAb staining (binds to conserved region of S2 subdomain). • No CR3022 binding was detected in cells transfected with the BA.4/BA.5 mRNA (mRNA-1273.045; davesomeran), while increased frequency and substantial binding was measured in cells transfected with mRNA contained in mRNA-1283. This indicates that the CR3022 mAb is specific to the original Wuhan-Hu-1 RBD and confirms that the S-2P encoded by the BA.4/BA.5 mRNA does not, as expected, contain the same binding epitope. • The frequency of cells that express the BA.4/BA.5 mRNA and the mRNA contained in mRNA-1283 was similar after staining with recombinant hACE-2. A small dose effect was observed overall. The MFI of expression showed similar results. <p>Cells that did not undergo transfection (mock-transfected cells) showed little to no change.</p>	

Figure 1. *In Vitro* Cell Surface Expression of BA.4/BA.5 SARS-CoV-2 S-2P After Transfection of Expi293 Cells With BA.4/BA.5 mRNA or mRNA-1283 (Positive Control) at 48 Hours

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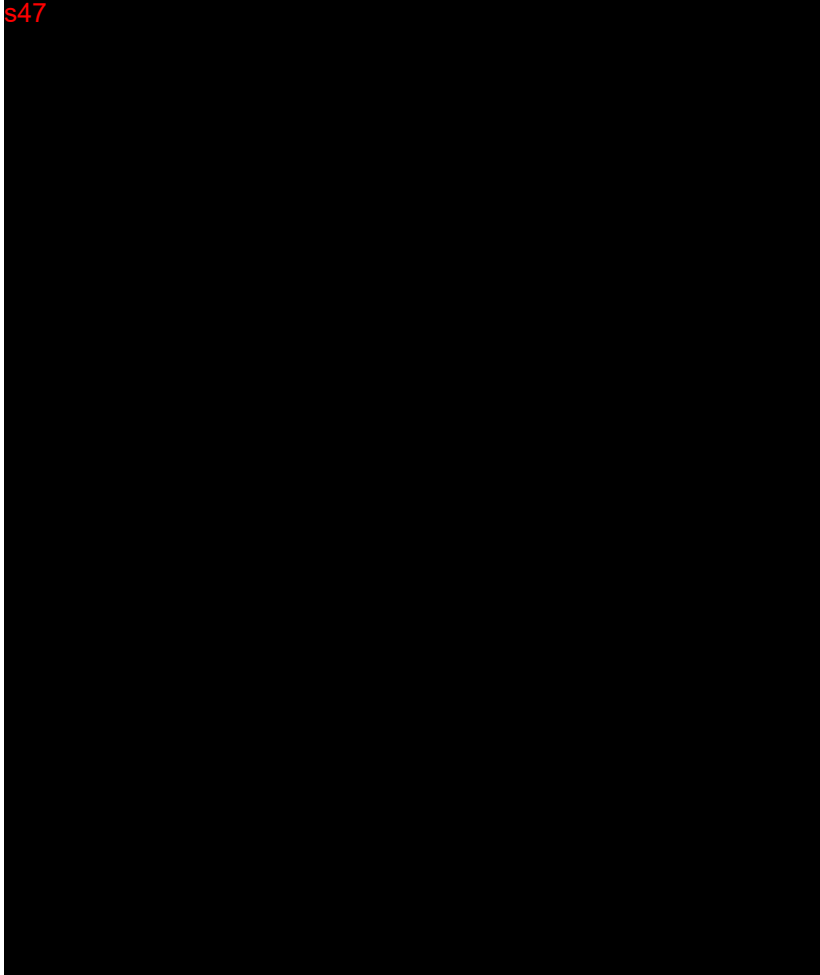
Abbreviations: hACE-2 = human angiotensin-converting enzyme 2; mAb = monoclonal antibody; MFI = mean fluorescence intensity; MFI*freq = the frequency of positive cells multiplied by the MFI; mRNA = messenger RNA; NTD = N-terminal binding; RBD = receptor binding domain; RBM = receptor binding motif; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

Notes: CR3022 mAb selectively binds to the original Wuhan-Hu-1 spike protein in the RBD. CC40.8 mAb is specific for the NTD of both the original Wuhan-Hu-1 spike as well as all variants that have emerged to date; in Figure 1B above, CC40.8 binds to a conserved region on the S2 subdomain. hACE-2 binds to the spike protein and was used as it will bind to the RBM in the original Wuhan-Hu-1 spike as well as all variants that have emerged to date. mRNA-1273.222 (bivalent vaccine) contains 2 S-2P mRNAs mixed in a 1:1 ratio: the mRNA found in the monovalent mRNA-1273 (Wuhan-Hu-1 S-2P spike sequence) and the mRNA found in the monovalent mRNA-1273.045 (Omicron subvariants BA.4/BA.5 S-2P spike sequence). mRNA-1283 (monovalent vaccine) contains mRNA that encodes the NTD and RBD of the Wuhan-Hu-1 spike protein linked together and inserted into the cell membrane with a transmembrane domain (NTD-RBD-HATM). Mock transfected meant that no mRNA was used during transfection.

1.2. IMMUNOGENICITY IN MICE

Table 2. Summary of immunogenicity of Omicron-matched mRNA vaccines as primary series in mice

Study details & Major findings																																
<i>Evaluation of Immunogenicity of Primary Series mRNA-1273.222 in BALB/c Mice. Study MOD-5482</i>																																
Study Design & Assay readout	<ul style="list-style-type: none"> The objective of this study was to investigate the immunogenicity potential elicited by a 2-dose primary vaccination series of the variant-modified bivalent ORIGINAL/OMICRON BA.4-5 mRNA-1273.222 vaccine in seventeen-week-old female BALB/c naïve mice. Study Design and Treatment Groups. Mice (n = 8/group) received 2 intramuscular (IM) injections of PBS control article or 1 µg mRNA vaccines as a primary series immunisation 3 weeks apart (Table below). Blood was collected from all animals on Day 21 (before second dose administered) and Day 35 (2 weeks after second dose). Serum samples were analysed for: <ul style="list-style-type: none"> bAb (binding antibody) responses via ELISA (enzyme-linked immunosorbent assay) nAb (neutralising antibody) responses via VSV-based (vesicular stomatitis virus) and lentivirus-based PSVNAs (pseudovirus neutralisation assays). Due to the lack of a standard SARS-CoV-2 neutralisation assay, both lentivirus-based and VSV-based PSVNAs were used in this study to capture the nAb response. <p><i>VSV-based (vesicular stomatitis virus) neutralisation assay:</i> Codon-optimized full-length spike genes (Wuhan-Hu-1 with D614G, BA.1, and BA.4/BA.5) were cloned into a pCAGGS vector. To generate recombinant VSVΔG-based SARS-CoV-2 pseudovirus, BHK-21/WI-2 cells were transfected with the spike expression plasmid and infected by VSVΔG-firefly-luciferase. Vero-E6 (ATCC; CRL-1586) cells were used as target cells for the neutralization assay.</p> <p><i>Lentivirus-based neutralisation assay:</i> Neutralisation activity against SARS-CoV-2 measured in a single-round-of-infection assay with lentivirus-based spike-pseudotyped virus particles (pseudoviruses). To produce SARS-CoV-2 pseudoviruses, an expression plasmid bearing codon-optimized SARS-CoV-2 full-length spike plasmid was co-transfected into HEK293T/17 cells (ATCC#CRL-11268) cells with packaging plasmid pCMVDR8.2, luciferase reporter plasmid pHR'CMV-Luc and a TMPRSS2 plasmid. Mutant spike plasmids were produced by Genscript. Pseudoviruses were mixed with 8 serial 4-fold dilutions of sera or antibodies in triplicate and then added to monolayers of angiotensin-converting enzyme 2-overexpressing 293T cells.</p>																															
	<table border="1"> <thead> <tr> <th rowspan="2">Group (n = 8/group)</th> <th colspan="3">Primary Series (Dose 1 and 2)</th> <th rowspan="2">Readouts</th> </tr> <tr> <th>Treatment (IM)</th> <th>Dose Level (µg)</th> <th>Dose Schedule</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>PBS Control</td> <td>-</td> <td rowspan="6">Day 1, 22</td> <td rowspan="6"> Serum (Day 21) Antibody responses (ELISA) Serum (Day 35): Antibody responses (ELISA and VSV- and lentivirus-based PSVNAs) </td> </tr> <tr> <td>2</td> <td>mRNA-1273</td> <td>1</td> </tr> <tr> <td>3</td> <td>mRNA-1273.529</td> <td>1</td> </tr> <tr> <td>4</td> <td>mRNA-1273.045</td> <td>1</td> </tr> <tr> <td>5</td> <td>mRNA-1273.214</td> <td>1</td> </tr> <tr> <td>6</td> <td>mRNA-1273.222</td> <td>1</td> </tr> </tbody> </table> <p>Note: ELISA measured specific bAb titers (S-2P of WA.1, BA.1 and BA.4/BA.5) and lentivirus-based, or VSV-based PSVNAs measured specific nAb titers (WA.1 [lentivirus-based PSVNA], WA.1 + D614G [VSV-based PSVNA] BA.1, and BA.4/BA.5).]</p>					Group (n = 8/group)	Primary Series (Dose 1 and 2)			Readouts	Treatment (IM)	Dose Level (µg)	Dose Schedule	1	PBS Control	-	Day 1, 22	Serum (Day 21) Antibody responses (ELISA) Serum (Day 35): Antibody responses (ELISA and VSV- and lentivirus-based PSVNAs)	2	mRNA-1273	1	3	mRNA-1273.529	1	4	mRNA-1273.045	1	5	mRNA-1273.214	1	6	mRNA-1273.222
Group (n = 8/group)	Primary Series (Dose 1 and 2)			Readouts																												
	Treatment (IM)	Dose Level (µg)	Dose Schedule																													
1	PBS Control	-	Day 1, 22	Serum (Day 21) Antibody responses (ELISA) Serum (Day 35): Antibody responses (ELISA and VSV- and lentivirus-based PSVNAs)																												
2	mRNA-1273	1																														
3	mRNA-1273.529	1																														
4	mRNA-1273.045	1																														
5	mRNA-1273.214	1																														
6	mRNA-1273.222	1																														
Test articles	<ul style="list-style-type: none"> Test articles were: <ul style="list-style-type: none"> Monovalent Vaccines <ul style="list-style-type: none"> (1) monovalent elasomeran RNA-1273 vaccine that contains a single mRNA encoding the SARS-CoV-2 S-2P antigen; (2) monovalent imelasomeran mRNA-1273.529 vaccine that contains a single mRNA encoding the SARS-CoV-2 S-2P antigen for BA.1 (S-2P.529 protein); (3) monovalent mRNA-1273.045 davesomeran vaccine that contains a single mRNA encoding the SARS-CoV-2 S-2P antigen of the BA.4/BA.5 subvariants of Omicron (S-2P.045 protein); Bivalent Vaccines <ul style="list-style-type: none"> (4) bivalent mRNA-1273.214 vaccine, which is a 1:1 bench side mix of separately formulated mRNA-1273 and mRNA-1273.529 imelasomeran vaccines; (5) bivalent mRNA-1273.222 vaccine, which is a 1:1 bench side mix of separately formulated mRNA-1273 and mRNA-1273.045 davesomeran vaccines. Formulation <ul style="list-style-type: none"> All mRNAs were formulated into a mixture of 4 lipids: SM-102, cholesterol, DSPC, and PEG2000-DMG. 																															

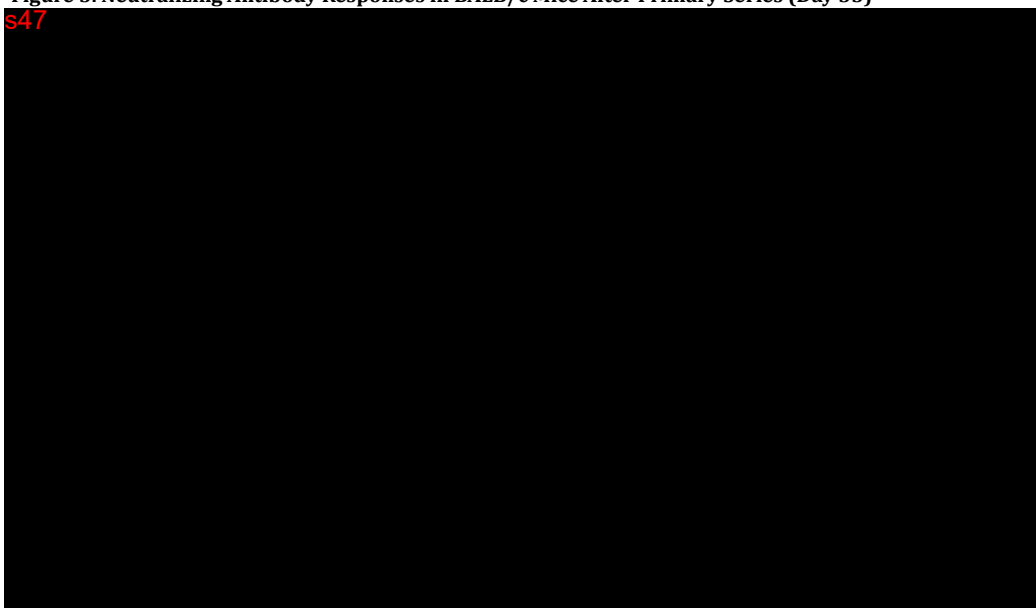
<p>Test Batches</p>		<p>Test Material</p>	<p>mRNA Description</p>	<p>mRNA Lot No(s).</p>	<p>LNP Formulation Lot No(s).</p>
		<p>mRNA-1273</p>	<p>mRNA SARS-CoV-2 spike protein^a</p>	<p>19150</p>	<p>26794.1</p>
		<p>mRNA-1273.529</p>	<p>mRNA SARS-CoV-2 BA.1 spike protein^b</p>	<p>19194</p>	<p>27783.1</p>
		<p>mRNA-1273.045</p>	<p>mRNA SARS-CoV-2 BA.4/BA.5 spike protein^c</p>	<p>1754876</p>	<p>28463.1</p>
		<p>mRNA-1273.214</p>	<p>mRNA SARS-CoV-2 spike protein + mRNA SARS-CoV-2 BA.1 spike protein (1:1)^d</p>	<p>19150 + 19194</p>	<p>NA</p>
		<p>mRNA-1273.222</p>	<p>mRNA SARS-CoV-2 spike protein + mRNA SARS-CoV-2 BA.4/BA.5 spike protein (1:1)^e</p>	<p>19150 + 1754876</p>	<p>NA</p>
<p>Results</p>	<p><u>Binding Ab by ELISA</u></p> <p>High binding antibody (IgG) titres against S-2P, S-2P.529, and S-2P.045 proteins seen after a 2-dose primary series with any monovalent or bivalent vaccines (Figure 2A-C). IgG titres markedly increased after the 2nd dose cf. the titres after the 1st dose.</p>				
	<p>Figure 2. Binding Antibody Responses in BALB/c Mice After Primary Series</p>  <p>Abbreviations: Ab = antibody; BA.1 = SARS-CoV-2 Omicron variant (B.1.1.529); BA.4/BA.5 = SARS-CoV-2 Omicron subvariants; GMT = geometric mean titre; IgG = immunoglobulin G; LLOQ = lower limit of quantification; mRNA = messenger RNA; PBS = phosphate-buffered saline; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; S-2P.045 = BA.4/BA.5 Omicron-specific S-2P; S-2P.529 = BA.1 Omicron-specific S-2P.</p> <p>Note: GMT (geometric mean titre) values are presented at the top of each figure, with red indicating the GMT for Day 21 (3 weeks after the first dose [i.e., post prime]) and blue indicating the GMT for Day 35 (2 weeks after the second dose [i.e., post boost]). Dotted line indicates LLOQ of the assays.</p>				

Neutralising Ab*VSV-based assay (Figure 3A):*

- On Day 35 (2 weeks after the second dose), high nAb against BA.4/BA.5 were detected in mice vaccinated with the bivalent mRNA-273.222 (19036) [elasomeran & davesomeran] or monovalent mRNA-1273.045 (13804) [davesomeran]. The nAb against BA.4/BA.5 were similar between the bivalent and monovalent vaccines, except for mRNA-1273 which was lower.
- nAb against WA.1 + D614G elicited by the bivalent mRNA-1273.222 vaccine was higher than that elicited by the monovalent mRNA-1273.045 vaccine (3035 cf. 110), but lower than by mRNA-1273.
- The bivalent mRNA-1273.214 [elasomeran & imelasomeran] and monovalent mRNA-1273.529 [imelasomeran] vaccines elicited high nAb against BA.1, but low nAb against BA.4/BA.5.
- The bivalent mRNA-1273.214 vaccine conferred much higher neutralisation against 'WA.1+ D614G' compared to the monovalent mRNA-1273.529 vaccine (8443 cf. 196).
- The monovalent mRNA-1273 vaccine showed a robust response against 'WA.1 + D614G' (16997), but a lower response against BA.1 (1025) and BA.4/BA.5 (111).

Figure 3. Neutralizing Antibody Responses in BALB/c Mice After Primary Series (Day 35)

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Abbreviations: Ab = antibody; GMT = geometric mean titer; ID₅₀ = inhibitory dilution 50%; LLOQ = lower limit of quantification; mRNA = messenger RNA; nAb = neutralizing antibody; PSVNA = pseudovirus neutralization assay; VSV = vesicular stomatitis virus.

Notes: Blue numbers and bars represent GMTs, and whiskers represent 95% confidence interval. Dotted line indicates LLOQ of the assays. In Figure A (VSV-based PSVNA), D614G = WA.1 + D614G.

Lentivirus-based PSVNAs (Figure 3B):

- Results (trends) of nAb response against WA.1, BA.1, and BA.4/BA.5 following 2-dose primary vaccination series of the monovalent mRNA-1273, bivalent mRNA-1273.214 and mRNA-1273.222 vaccines were similar to those observed using the VSV-based assay (Figure 3A).
- On Day 35 (2 weeks after the second dose), mice vaccinated with the bivalent mRNA-1273.222 showed a robust nAb response against BA.4/BA.5, while the response against WA.1 and BA.1 was low.
- Bivalent mRNA-1273.214 vaccine induced robust nAb response against BA.1, and a lesser response against BA.4/BA.5. nAb against WA.1 was similar to that induced by mRNA-1273.
- Monovalent mRNA-1273 vaccine elicited high neutralization against WA.1 and low responses against BA.1 and BA.4/BA.5.

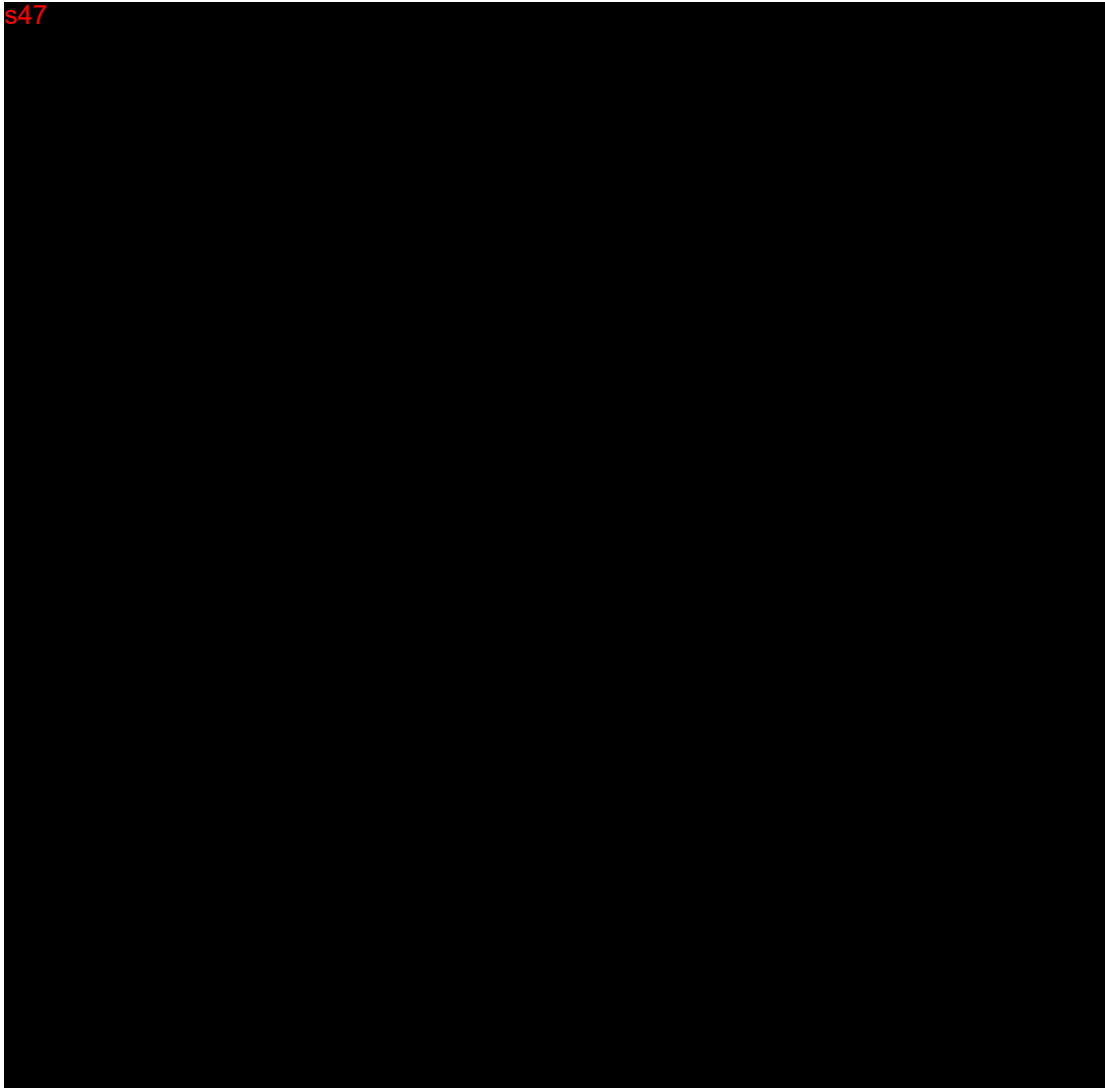
Conclusion	<ul style="list-style-type: none"> Robust S-2P, S-2P.529, S-2P.045 IgG GMTs were seen in all mRNA groups 2 weeks after second dose. Monovalent BA.1-matched mRNA-1273.529 and BA.4/BA.5-matched mRNA-1273.045 vaccines showed low nAb when assessed against non-matched spike antigens. Bivalent mRNA-1273.222 and mRNA-1273.214 vaccines elicited high neutralizing nAb against BA.4/BA.5 and BA.1 pseudoviruses, respectively, while mRNA-1273 induced very low nAb against the omicron variants. WA.1 nAb elicited by the bivalent mRNA-1273.214 vaccine were similar to that elicited by monovalent mRNA-1273 vaccine, demonstrating greater neutralisation breadth of mRNA-1273.214 than mRNA-1273. The bivalent mRNA-1273.222 elicited lower WA.1 nAb than the monovalent mRNA-1273, suggesting mRNA-1273.222 may be less effective than mRNA-1273 in the protection against infection by the original strain. <p><i>Overall, the bivalent mRNA-1273.222 vaccine induces high nAb against Omicron BA.4/5, but relatively low nAb against WA.1.</i></p>
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1.3. IMMUNOGENICITY AND PROTECTION

Table 3. Summary of immunogenicity/protection after Primary Series Vaccination with mRNA-1273

Study details & Major findings																																																			
<p><i>Evaluation of Immunogenicity and Protection From a Booster Dose of the mRNA-1273.222 Vaccine After Primary Series Vaccination With mRNA-1273 in Mice. Study WASHU-K18-89</i></p> <p>Note: This study was also published (Scheaffer et al., 2022¹) - additional data from this reference is presented below where indicated.</p>																																																			
Study Design	<ul style="list-style-type: none"> Study Design and Treatment Groups (see Table below) <p>Mice (K18-hACE2; n = 8-10/group). mRNA vaccines given IM in 50 µL of PBS in hind leg at a dose of 0.25 µg.</p>																																																		
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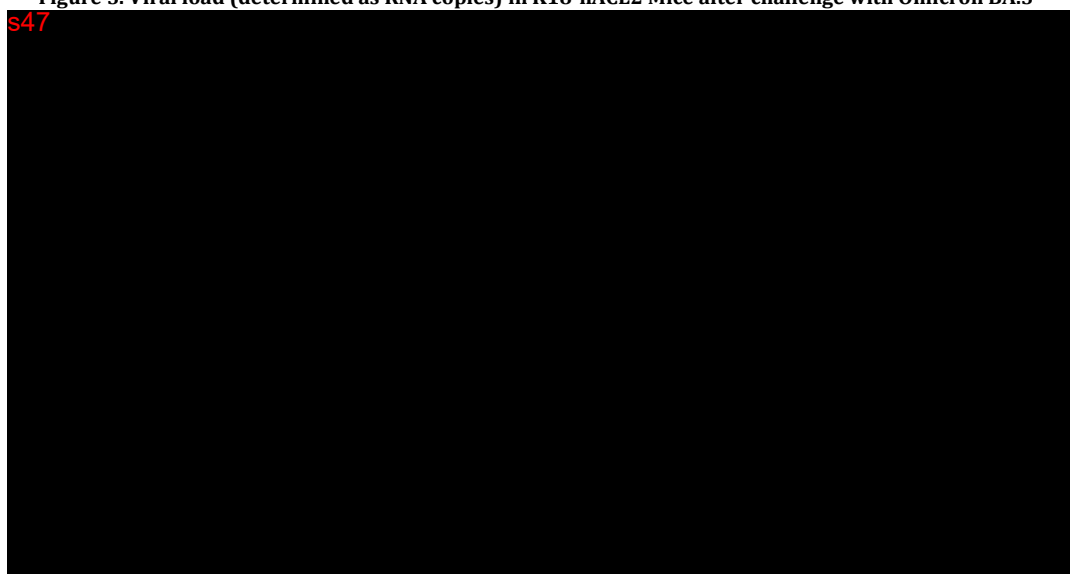
¹ Scheaffer S.M., Lee D., Whitener B., Ying B., Wu K., Liang C.Y. et al. (2022) Bivalent SARS-CoV-2 mRNA vaccines increase breadth of neutralization and protect against the BA.5 Omicron variant in mice. *Nat. Med.* <https://doi.org/10.1038/s41591-022-02092-8> [ePub ahead of print].

Study details & Major findings	
Assays	<ul style="list-style-type: none"> ○ Focus reduction neutralization test (FRNT): Serial dilutions of sera incubated with 10² FFU of WA.1/2020 + D614G, BA.1, or BA.5. Antibody-virus complexes added to Vero-TMPRSS2 cell monolayers. ○ Viral burden measured using quantitative reverse transcription polymerase chain reaction
Omicron Challenge	<ul style="list-style-type: none"> ● BA.5 isolate was used for the challenge by the intranasal route, isolated in California (hCoV-19/USA/CA-Stanford- 79_S31/2022). All viruses subjected to next-generation sequencing to confirm the introduction and stability of substitutions.
Results	
Neutralising antibodies	
<ul style="list-style-type: none"> ○ Pre-boost nAb against WA.1/2020 + D614G and Delta B.1.617.2 were similar in all groups (Figure 4B and 4C), with no neutralising activity against BA.1 or BA.5 at the 1/60 limit of detection (Figure 4D and 4E). ○ After boosting with mRNA-1273, serum nAb against WA.1/2020 + D614G and Delta B.1.617.2 were increased (by ~4 fold) cf. with pre-boost nAb (Figure 4B and 4C), with very small or no increases in nAb against BA.1 and BA.5 (Figure 4D and 4E). ○ Boosting with either mRNA-1273.214 or mRNA-1273.222 generally induced nAb against WA.1/2020 + D614G and Delta similar to or slightly higher than after boosting with mRNA-1273 (Figure 4B and 4C). ○ A booster dose of mRNA-1273.214 or mRNA-1273.222 induced nAb against BA.5 and BA.1, although the nAb against the Omicron variants were much lower than nAb against WA1 or Delta (~6-20% of the nAb against WA1). Interestingly, mRNA-1273.214 induced nAb against BA.5 similar to mRNA-1273.222. 	
<p>Figure 4. Neutralising Antibody Responses in K18-hACE2 Mice before and after a booster dose of mRNA-1273, mRNA-</p> 	
<p>Abbreviations: BA.1 = SARS-CoV-2 Omicron variant (B.1.1.529); BA.5 = SARS-CoV-2 Omicron subvariant; EC₅₀ = half-maximal effective concentration; ns = not significant; S = phosphate-buffered saline. *p < 0.05; ** p < 0.01. Note: Serum neutralisation analysis used a limit of detection (LOD) of 1:60 dilution</p>	

Protection

- The viral load (RNA levels) in lungs, nasal turbinates, and nasal wash in mice boosted with mRNA-1273, mRNA-1273.214 or mRNA-1273.222 was markedly lower than in mice boosted with PBS only or mice primed and boosted with UNFIX-01 control (Figure 5).
- The viral load in lung in mice boosted with mRNA-1273.222 was lower than in mice boosted with mRNA-1273 (Figure 5A), but the nasal and nasal turbinate viral load was similar in mice boosted with the monovalent and bivalent vaccines (Figure 5B,C).
- Overall, all 3 vaccines conferred protection against infection based on viral load and the bivalent vaccines were more effective than the original monovalent vaccine based on lung viral load.

Figure 5. Viral load (determined as RNA copies) in K18-hACE2 Mice after challenge with Omicron BA.5



Four weeks after boosting with mRNA-1273, mRNA-1273.214, mRNA-1273.222, or control (PBS) vaccines, mice were challenged with Omicron BA.5, and viral load measured in the upper and lower respiratory tract. p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001

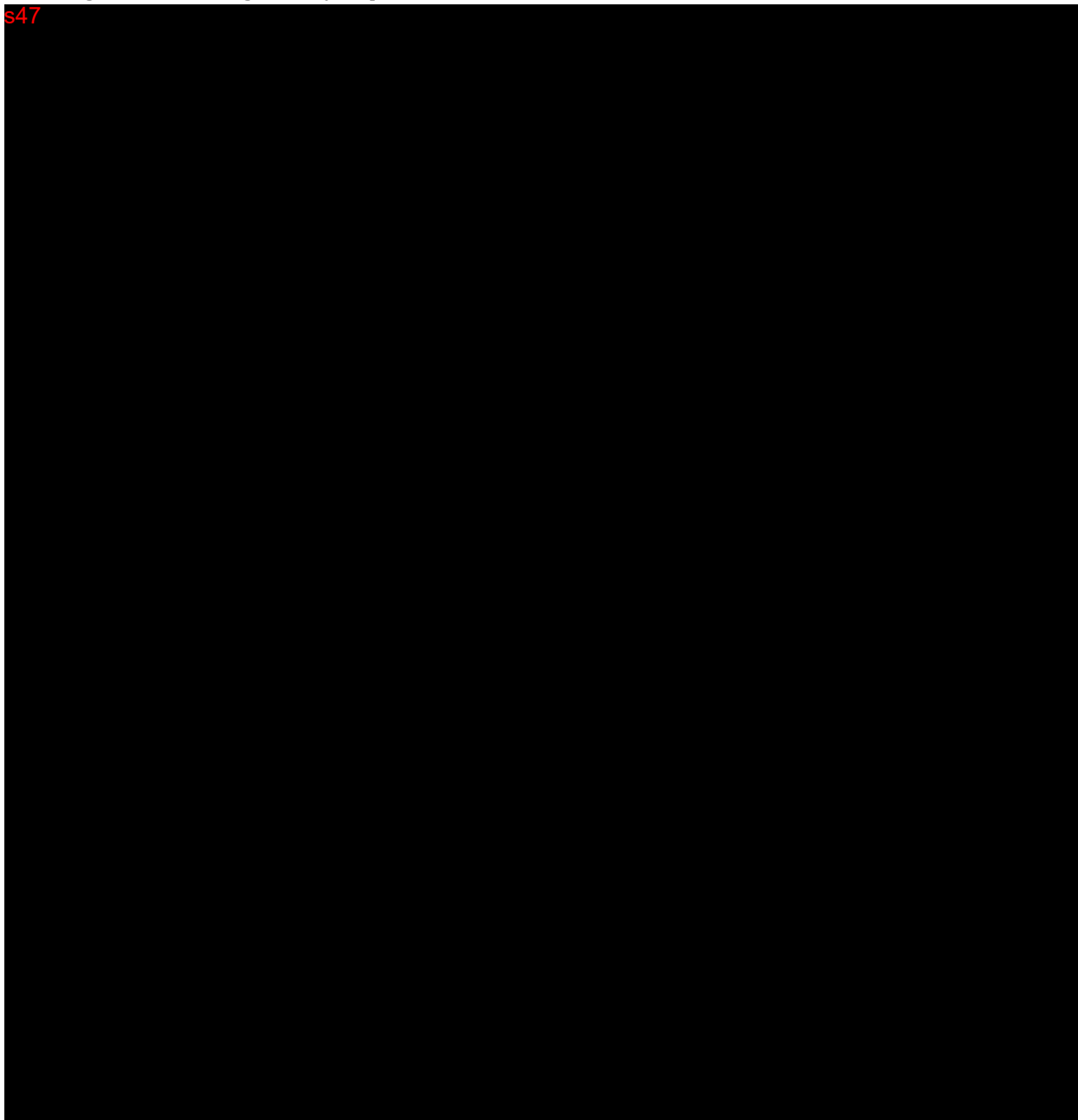
Study Limitation	A low dose (0.25 µg) was used for the primary series immunization and for the booster to allow for the possibility of breakthrough infection after a 3-dose vaccination series, to enable measurement of different levels of protection in mice given the monovalent mRNA-1273 vaccine versus the 2 bivalent vaccines (mRNA-1273.214 and mRNA-1273.222). A higher booster could have elicited higher nAb responses but would likely have driven increased levels of heterologous protection, as has been seen in other mouse challenge studies (Ying <i>et al.</i> , 2022 ²).
Conclusion	<ul style="list-style-type: none"> • Dosing with the mRNA-1273.222 [elasomeran + davesomeran] vaccine as a booster following a primary series of the mRNA-1273 [elasomeran] vaccine enhanced nAbs against both BA.1 and BA.5. • The enhanced nAb production after boosting with the mRNA-1273.222 vaccine against BA.1 and BA.5 evident cf. boosting with the mRNA-1273 vaccine. • Comparable immunogenicity and protection findings in mice boosted with the mRNA-1273.214 (elasomeran [mRNA-1273] and imelasomeran [mRNA-1273.529]) vaccine with mRNA-1273.222 vaccine following a primary series vaccination with mRNA-1273. • Overall, boosting with either mRNA-1273.222 or mRNA-1273.214 vaccine enhanced protection against BA.5 infection cf. with protection elicited by boosting with the mRNA-1273 vaccine.

² Ying B, Scheaffer SM, Whitener B, Liang CY, Dmytrenko O, Mackin S, *et al.* (2022) Boosting with variant-matched or historical mRNA vaccines increases protection against SARS-CoV-2 Omicron infection in mice. *Cell*;185(9):1572-87.e11

The above study was also published (*Scheaffer et al., 20022*).
Additional data from the published article is summarised below.

Figure 6. Neutralising Antibody Responses in K18-hACE2 Mice before and after a booster dose of mRNA-1273,

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- mRNA-1273 series + PBS
- mRNA-1273 series + mRNA-1273 (Wuhan-1)
- mRNA-1273 series + mRNA-1273.214 (Wuhan-1/BA.1)
- mRNA-1273 series + mRNA-1273.222 (Wuhan-1/BA.4/5)

Extended Data Fig. 5 | Comparison of serum neutralization of authentic WA1/2020 D614G, B.1.617.2, BA.1, and BA.5 viruses before and after boosting. Seven-week-old female K18-hACE2 mice were immunized with two sequential 0.25 µg doses of control mRNA or mRNA-1273 and then boosted 31 weeks later with PBS, 0.25 µg of control mRNA, or 0.25 µg mRNA-1273, mRNA-1273.214, or mRNA-1273.222. Paired analysis of pre- and post-boost serum neutralizing titers against WA1/2020 D614G, B.1.617.2, BA.1 and BA.5 from samples obtained from

animals (data from Fig. 3) (n = 8 for mRNA-1273.214 booster, n = 9 for control mRNA and mRNA-1273 booster, n = 10 for PBS and mRNA-1273.222 booster, two experiments. GMT values are indicated at the top of the graphs, dotted lines show the LOD based on a 1/60 (WA1/2020 D614G, B.1.617.2) or 1/30 serum dilution (BA.1, BA.5). Statistical analysis: two-tailed Wilcoxon signed-rank test (exact *P* values are indicated except for *P* > 0.05, not significant (ns)). Primary data are provided as a Source Data file.

Infectious viral load in the lung

Analysis of infectious virus in the lung at 4 days post-infection using plaque assays showed substantial reductions in viral burden in animals boosted with mRNA-1273-214 or mRNA-1273.222 vaccines compared to those receiving a control vaccine or immunized with two doses of mRNA-1273 and boosted with PBS or mRNA-1273 (Figure 6).

Cytokine and chemokine levels in the lung

As another gauge of vaccine-induced protection, cytokine and chemokine levels were measured in the lung of the BA.5-challenged K18-hACE2 mice at 4 days post-infection using a multiplexed assay. Mice immunised with the control vaccine (UNFIX-01) or those receiving a primary vaccination series with mRNA-1273 and a booster dose of PBS showed higher levels of many inflammatory cytokines and chemokines in lung homogenates than unvaccinated, unchallenged (naive) animals (Figure 7). In comparison, the lungs of BA.5-challenged mice that were boosted with mRNA-1273, mRNA-1273-214 or mRNA-1273.222 vaccines showed substantially lower or undetectable levels of most pro-inflammatory cytokines and chemokines. For several cytokines and chemokines (for example, IFN- γ , IL-6, CXCL9 and CXCL10), lower levels were observed after boosting with mRNA-1273-214 and mRNA-1273.222, but not mRNA-1273, compared to boosting with PBS. Consistent with the virological data, protection against BA.5-induced lung inflammation was increased by boosting with mRNA vaccines, with a modest (albeit not statistically significant), improvement after administration of bivalent vaccines compared to the parental monovalent mRNA vaccine.

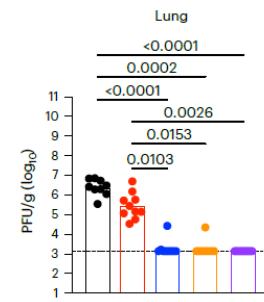


Figure 7 Infectious viral load in lung (see legend and notes for Figure 5)

Figure 8 Cytokine and chemokine levels in lung homogenates at 4 days post-infection

S47

● Control mRNA series ● mRNA-1273 series + PBS ● mRNA-1273 series + mRNA-1273 (Wuhan-1)
 ● mRNA-1273 series + mRNA-1273.214 (Wuhan-1/BA.1) ● mRNA-1273 series + mRNA-1273.222 (Wuhan-1/BA.4/5)

Data expressed as fold change relative to naive mice (\log_2 values plotted). $n = 8$ for mRNA-1273.214, $n = 9$ for control mRNA and mRNA-1273, $n = 10$ for PBS and mRNA-1273.222 and $n = 4$ for naive, two experiments; lines illustrate median values, and dotted lines indicate LOD for each respective analyte.

Histological analysis of lung tissues

Lung sections were histologically examined 4 days post-infection in BA.5-challenged mice:

- Mice immunised with the control vaccine or those receiving a primary mRNA-1273 vaccination series with a booster of PBS showed patchy, bridging immune cell infiltration, air way space thickening and alveolar congestion (Figure 8). The pathology seen after BA.5 infection of K18-hACE2 mice, however, was less than with historical or other variant SARS-CoV-2 strains as reported for other Omicron strains.
- BA.5-challenged mice that had been boosted with mRNA-1273 had more limited, focal immune cell infiltration in airspaces, indicating the monovalent vaccine conferred some protection against BA.5.
- Mice boosted with mRNA-1273.214 or mRNA-1273.222 vaccines showed virtually no lung pathology, similar to uninfected control mice.

Figure 9. Lung pathology of K18-hACE2 mice from BA.5 challenge

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Hematoxylin and eosin staining of lung sections harvested at 4 days post-infection from mice immunised with control mRNA (CTRL-mRNA) or mRNA-1273 (primary two dose series) and then boosted with PBS, mRNA-1273, mRNA-1273.214 or mRNA-1273.222 vaccines. An uninfected (naive) animal is shown for comparison.

Low (top: scale bars, 1 mm), moderate (middle: scale bars, 200 µm) and high (bottom: scale bars, 50 µm) power images are shown. Representative images of multiple lung sections from n = 2 for each group, two experiments.

Overall, these findings are consistent with the viral burden and cytokine and chemokine reductions and demonstrate protection against SARS-CoV-2 infection and lung injury after boosting with either bivalent mRNA vaccines, and to a lesser extent, by the monovalent vaccine.



Nonclinical Evaluation Report

Andusomeran [SPIKEVAX XBB.1.5 COVID-19 VACCINE]

Submission No: PM-2023-03611-1-2

Sponsor: Moderna Australia Pty Ltd

September 2023

TGA Health Safety
Regulation

A decorative graphic at the bottom of the page consisting of several overlapping, wavy bands in shades of blue and green, creating a modern, flowing design.

NONCLINICAL EVALUATION REPORT

Submission type: New vaccine

Sponsor: Moderna Australia Pty Ltd

Generic name: Andusomeran

Trade name: SPIKEVAX XBB.1.5 COVID-19 VACCINE

Dose form and strength: Injection, Suspension; 100 µg in 1 mL

Drug class: mRNA encapsulated in LNP

Submission No: PM-2023-03611-1-2

Tox file No: E23-262971

TRIM reference: D23-2032701

Date authorised: 1 September 2023

Note: This evaluation report has been peer-reviewed and is authorised for release to the sponsor.

TABLE OF CONTENTS

SUMMARY, ASSESSMENT AND RECOMMENDATIONS	4
PRODUCT INFORMATION	5
ROUND 1 EVALUATION — MILESTONE 3.....	5
MAIN BODY OF REPORT	8
1. INTRODUCTION	8
1.1. BACKGROUND	8
1.2. OVERSEAS REGULATORY STATUS.....	9
1.3. SCOPE OF NONCLINICAL DATA	9
2. PRIMARY PHARMACODYNAMICS	9
2.1. IMMUNOGENICITY IN MICE	9
2.1.1. Primary series of monovalent and bivalent SARS-CoV-2 XBB.1.5-containing vaccines	9
2.1.2. Monovalent & bivalent SARS-CoV-2 XBB.1.5-containing vaccine boosters.....	12
2.1.3. Monovalent & bivalent SARS-CoV-2 XBB.1.16-containing vaccine boosters.....	15

SUMMARY, ASSESSMENT AND RECOMMENDATIONS

- Moderna Australia Pty Ltd has applied for registration/submitted data in support of a new COVID-19 vaccine (SPIKEVAX XBB.1.5; mRNA-1273.815), containing andusomeran in lipid nanoparticles (LNP). The SPIKEVAX XBB.1.5 COVID-19 vaccine is indicated for active immunisation to prevent coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 in individuals 6 years of age and older. The vaccine dose is one dose of 0.25 mL (6 - <12 years old) and 0.5 mL (above 12 years), containing 25 and 50 µg of mRNA, respectively, given intramuscularly (IM) at least 3 months after the most recent dose of a COVID-19 vaccine.
- The new monovalent vaccine (SPIKEVAX XBB.1.5) is manufactured using the same mRNA platform and manufacturing method as the provisionally approved Original/Omicron BA.1 bivalent vaccine.
- Module 4 comprised of 3 *in vivo* pharmacology studies conducted in BALB/c mice. The submitted studies evaluated immunogenicity of XBB-containing mRNA vaccines given as a primary series, or as a booster dose following primary series vaccination with mRNA-1273. The route of administration of the mRNA vaccines used in the nonclinical *in vivo* pharmacology studies was IM, consistent with the clinical route. No T cell characterisation and pharmacology protection studies for XBB.1.5 were submitted. This is acceptable as there are no changes to the original vaccine formulation except for replacement of a serotype strain RNA. Cellular responses and proof of protection from an *in vivo* challenge with SARS CoV-2 virus, were characterised previously with the initial Spikevax vaccine.
- A 2-dose primary series vaccination with the monovalent mRNA-1273.815 and bivalent mRNA-1273.231 induced high S-2P binding antibody (bAb) titres and elicited high neutralising antibodies (nAb) against the Omicron subvariant XBB strains, XBB.1.5 and XBB.1.16. The neutralisation titres were >45-fold higher than those elicited by monovalent mRNA-1273.045 or bivalent mRNA-1273.222. Compared to the monovalent mRNA-1273.815, the bivalent mRNA-1273.231 had higher titres against the ancestral and BA.4/BA.5 strains (due to the inclusion of BA.4/BA.5 in the bivalent vaccine). Elicited nAb titres against XBB.1.5 and XBB.1.16 were comparable, indicating that the two strains are antigenically similar.
- Monovalent mRNA-1273.815 or bivalent mRNA-1273.231 as a booster following a primary series of mRNA-1273 vaccination induced high S-2P bAb titres and high nAb responses against the Omicron subvariant XBB strains, XBB.1.5 and XBB.1.16. Higher nAb response was achieved with mRNA-1273.815 than mRNA-1273.231. mRNA-1273.231 had higher titres against ancestral and BA.4/BA.5 strains compared with mRNA-1273.815 (due to the inclusion of BA.4/BA.5 in the bivalent vaccine). Comparable nAb titres were observed for against XBB.1.5 and XBB.1.16, indicating that the two strains are antigenically similar.
- Monovalent mRNA-1273.116 or bivalent mRNA-1273.234 as a booster following a primary series of mRNA-1273 vaccination induced high S-2P bAb titres as well as neutralizing titres against the Omicron subvariant XBB strains, XBB.1.16 and XBB.1.5.
- Overall, boosting with mRNA-1273.815 enhanced IgG responses and neutralising activity against Omicron subvariant XBB strains compared to boosting with mRNA-1273.
- No toxicity studies were submitted. This is acceptable since the new mRNA (andusomeran) uses the same backbone and manufacture platform as elasomeran and imelasomeran, with no changes to vaccine formulation except for the additional mRNA.
- There are no nonclinical objections to the provisional approval of the SPIKEVAX XBB.1.5 vaccine.
- The draft Product Information should be amended as directed on pages 5–7.

PRODUCT INFORMATION

ROUND 1 EVALUATION — MILESTONE 3

The following comments refer to the draft Product Information document (SPIKEVAX XBB.1.5_PI_v0.2_Aug 2023) accompanying the sponsor's letter of application dated 15 August 2023. Where changes are suggested, text proposed to be inserted is underlined and text to be deleted is shown struck-through (highlighted in yellow below).

4.5 INTERACTIONS WITH OTHER MEDICINES AND OTHER FORMS OF INTERACTIONS

The proposed statements are the same as in the previously approved Product Information for the bivalent vaccine and is acceptable:

“No interaction studies have been performed. Concomitant administration of SPIKEVAX (original), SPIKEVAX BIVALENT ORIGINAL/OMICRON (BA.1), SPIKEVAX BIVALENT ORIGINAL/OMICRON BA.4-5 or SPIKEVAX XBB.1.5 with other vaccines has not been studied.”

4.6 FERTILITY, PREGNANCY AND LACTATION

Effects on fertility

The proposed text is the same as in the previously approved Product Information for the bivalent vaccine and is acceptable:

“Animal studies with SPIKEVAX (original) do not indicate direct or indirect harmful effects with respect to reproductive toxicity in females.

In a combined fertility and developmental toxicity study, 100 micrograms of mRNA (elasomeran) and other ingredients included in a single human dose of SPIKEVAX (original) was administered to female rats by the intramuscular route on four occasions: 28 and 14 days prior to mating, and on gestation days 1 and 13. SARS-CoV-2 antibody responses were present in dams from prior to mating to the end of the study on lactation day 21 as well as in fetuses and offspring. There were no vaccine-related adverse effects on female fertility, pregnancy, embryofetal or offspring development or postnatal development. No data are available on SPIKEVAX (original), SPIKEVAX BIVALENT ORIGINAL/OMICRON (BA.1), SPIKEVAX BIVALENT ORIGINAL/OMICRON BA.4-5 or SPIKEVAX XBB.1.5 vaccine placental transfer or excretion in milk. The effect on male fertility has not been determined.”

Use in pregnancy

The sponsor proposes Pregnancy Category B and the following statement:

“A large amount of observational data from pregnant women vaccinated with SPIKEVAX (original) during the second and third trimester has not shown an increase in adverse pregnancy outcomes. While data on pregnancy outcomes following vaccination during the first trimester are presently limited, no increased risk for miscarriage has been seen. Animal studies do not indicate direct or indirect harmful effects with respect to pregnancy, embryo/foetal development, parturition or postnatal development (see Effects on fertility). SPIKEVAX XBB.1.5 can be used during pregnancy.”

The proposed Pregnancy Category B is considered appropriate for this product. The proposed statement of which the nonclinical statements are same as in the previously approved Product Information for the bivalent vaccine and is acceptable. Corrected spelling for fetal and a minor editorial change are recommended. Thus:

“A large amount of observational data from pregnant women vaccinated with SPIKEVAX (original) during the second and third trimester has not shown an increase in adverse pregnancy outcomes. While data on pregnancy outcomes following vaccination during the first trimester are presently limited, no increased risk for miscarriage has been seen. Animal studies do not indicate direct or indirect harmful effects with respect to pregnancy, embryo/foetal development, parturition or postnatal development (see [4.6 Fertility, pregnancy and lactation](#), Effects on fertility). SPIKEVAX XBB.1.5 can be used during pregnancy.”

Use in lactation

The proposed text requires reviewing by the clinical evaluator:

“No effects on the breastfed newborn/infant are anticipated since the systemic exposure of the breastfeeding woman to SPIKEVAX XBB.1.5 is negligible. Observational data from women who were breastfeeding after vaccination have not shown a risk for adverse effects in breastfed newborns/infants. SPIKEVAX XBB.1.5 can be used during breastfeeding.”

5.1 PHARMACODYNAMIC PROPERTIES

Mechanism of action

The proposed text is the same as in the previously approved Product Information for the bivalent vaccine and is acceptable:

“SPIKEVAX (original) (elasomeran) and SPIKEVAX BIVALENT ORIGINAL/OMICRON (BA.1) (elasomeran/imelasomeran) both contain mRNA encapsulated in lipid nanoparticles. The mRNA encodes for the full-length SARS-CoV-2 spike protein modified with 2 proline substitutions within the heptad repeat 1 domain (S-2P) to stabilise the spike protein into a prefusion conformation. After intramuscular injection, cells at the injection site and the draining lymph nodes take up the lipid nanoparticle, effectively delivering the mRNA sequence into cells for translation into viral spike protein. The delivered mRNA does not enter the cellular nucleus or interact with the genome, is nonreplicating, and is expressed transiently mainly by dendritic cells and subcapsular sinus macrophages. The expressed, membrane-bound spike protein of SARS-CoV-2 is then recognised by immune cells as a foreign antigen. This elicits both T-cell and B-cell responses to generate neutralising antibodies, which may contribute to protection against COVID-19.

The nucleoside-modified mRNA in SPIKEVAX BIVALENT ORIGINAL/OMICRON BA.4-5 (elasomeran/davesomeran) and in SPIKEVAX XBB.1.5 (andusomeran) is formulated in lipid particles, which enable delivery of the nucleoside-modified mRNA into host cells to allow expression of the SARS-CoV-2 S antigen. The vaccine elicits an immune response to the S antigen, which protects against COVID-19.”

5.2 PHARMACOKINETIC PROPERTIES

The following statements on the pharmacokinetics are the same as in the previously approved Product Information for the bivalent vaccine and is acceptable:

“Not applicable.”

5.3 PRECLINICAL SAFETY DATA

The statements on preclinical safety data are the same as in the previously approved Product Information for the bivalent vaccine and is acceptable:

“Non-clinical data reveal no special hazard for humans based on conventional studies of repeat dose toxicity and reproductive and developmental toxicity. The full relevance of animal studies to human risk with vaccines for COVID-19 remains to be established.”

Genotoxicity

The statements on genotoxicity are generally same as in the previously approved Product Information for the bivalent vaccine and are acceptable. Vaccine names have been included to maintain consistency with the Product Information of the previously approved bivalent vaccine:

“The novel lipid components SM-102 and PEG-2000-DMG of SPIKEVAX (original), SPIKEVAX BIVALENT ORIGINAL/OMICRON (BA.1), SPIKEVAX BIVALENT ORIGINAL/OMICRON BA.4-5 and SPIKEVAX XBB.1.5 the vaccine were negative in the bacterial reverse mutation Ames test and in vitro micronucleus test in human peripheral blood lymphocytes. A luciferase mRNA in SM102-containing lipid nanoparticles was negative in a rat bone marrow micronucleus assay (IV dose of SM-102 28.5 mg/kg, PEG-2000-DMG 2.8 mg/kg), whilst a surrogate ZIKA mRNA-based vaccine formulated in SM-102-containing lipid nanoparticles induced micronuclei in male rats, but not in females (IV dose of SM-102 60 mg/kg, PEG-2000-DMG 6 mg/kg). The weight of evidence suggests the genotoxicity potential of the novel lipid components SM-102 and PEG-2000-DMG is very low. The other components of SPIKEVAX (original), SPIKEVAX BIVALENT ORIGINAL/OMICRON (BA.1), SPIKEVAX BIVALENT ORIGINAL/OMICRON BA.4-5 or SPIKEVAX XBB.1.5 the vaccine (other lipids and mRNA) are not expected to be genotoxic.”

Carcinogenicity

The statements on carcinogenicity are the same as in the previously approved Product Information for the bivalent vaccine and are acceptable:

“Carcinogenicity studies were not performed. The components of the vaccine (lipids and mRNA) are not expected to have carcinogenic potential.”

MAIN BODY OF REPORT

Rationale

The development of Omicron-containing vaccine boosters, specifically mRNA-1273.214 (BA.1-containing bivalent) and mRNA-1273.222 (BA.4/BA.5-containing bivalent) enhanced protection against the early Omicron strains. The Omicron virus family has continued to rapidly evolve with new subvariants (XBB.1.5, XBB.1.9.1, and XBB.1.16 emerged in early 2023) with additional growth advantages, increased transmissibility, and the ability to escape authorized BA.1- or BA.4/BA.5-containing bivalent booster vaccine- or infection-derived immunity. Variant-modified vaccines are considered to provide optimal protection as new SARS-CoV-2 variants of concern (VOCs) emerge. SPIKEVAX XBB.1.5 (andusomeran) is a new monovalent XBB strain-containing vaccine likely to substantially boost protection against XBB subfamily strains including XBB.1.5 or XBB.1.16.

1. INTRODUCTION

1.1. BACKGROUND

Moderna Australia Pty Ltd proposes to register a new monovalent strain update for SPIKEVAX XBB.1.5 COVID-19 vaccine, which integrates the mRNA sequence encoding for the pre-fusion stabilized spike protein of the XBB.1.5 (Omicron) Variant-of-Interest in mRNA CX-038839. The lipid nanoparticle (LNP) (same as the currently approved Spikevax products) formulation delivery system (containing SM-102, cholesterol, DSPC and PEG2000-DMG lipids) is used to encapsulate the CX-038839 mRNA into the new mRNA-1273.815 [REDACTED]-B. All mRNA-1273 LNP have been manufactured utilizing the same manufacturing process and controls pre-established for the mRNA-1273 LNPs platform (Module 2.3.S, Drug substance quality overall summary).

SPIKEVAX XBB.1.5 COVID-19 vaccine suspension for injection is formulated as follows:

Table 1.1. Product formulation (Single-Dose Vial)

Ingredient	Function	Quantity		
		mg/mL	mg/vial (0.65 mL fill)	mg/dose (0.50 mL dose)
Total RNA	Active			
SM-102	LNP Individual lipids making up the LNP			
Cholesterol				
DSPC				
PEG2000-DMG				
Trometamol (Tris)	Components for Tris buffer			
Trometamol-HCl (Tris-HCl)				
Acetic acid (Glacial)	Components from acetate buffer in LNP			
Sodium acetate trihydrate				
Sucrose	Cryoprotectant			
Water for Injection	Solvent			

DSPC = 1,2-distearoyl-sn-glycero-3-phosphocholine; PEG2000-DMG = polyethylene glycol 2000 dimyristoyl glycerol; pH 5.47

* Comprises 0.017 mg sodium

Table 1.2. Product formulation (Pre-filled Syringe)

Ingredient	Function	Quantity		
		mg/mL	mg/dose (0.50mL fill)	mg/dose (0.25 mL dose)
Total RNA	Active	s47		
SM-102	LNP	Individual lipids making up the LNP		
Cholesterol				
DSPC				
PEG2000DMG				
Trometamol (Tris)	Components for Tris buffer			
Trometamol-HCl (Tris-HCl)				
Acetic acid (Glacial)	Components from acetate buffer in LNP			
Sodium acetate trihydrate				
Sucrose	Cryoprotectant			
Water for Injection	Solvent			

DSPC = 1,2-distearoyl-sn-glycero-3-phosphocholine; PEG2000-DMG = polyethylene glycol 2000 dimyristoyl glycerol; pH

* Comprises 0.017 mg sodium; ** Comprises 0.0083 mg sodium

1.2. OVERSEAS REGULATORY STATUS

A similar application has been made in Canada (June 2023), Switzerland (July 2023), Singapore (planned August 2023), EU (June 2023) and in the USA (June 2023).

1.3. SCOPE OF NONCLINICAL DATA

The Sponsor has submitted nonclinical studies to evaluate immune responses generated by the new proposed vaccine (expressing updated variant spike components) against antigenically distinct circulating virus variants. The studies were conducted with the following XBB-containing vaccines:

- **Monovalent mRNA-1273.815 vaccine** - A single mRNA encoding the SARS-CoV-2 S-2P antigen of the XBB.1.5/XBB.1.9.1 subvariant of Omicron (Note: the spike protein of XBB.1.9.1 is identical to that of XBB.1.5)
- **Monovalent mRNA-1273.116 vaccine** - A single mRNA encoding the SARS-CoV-2 S-2P antigen of the XBB.1.16 subvariant of Omicron
- **Bivalent mRNA-1273.231 vaccine** - Co-formulation of the mRNA-1273.045 vaccine (monovalent vaccine containing a single mRNA encoding the SARS-CoV-2 S-2P antigen of the BA.4/BA.5 subvariants of Omicron) and the mRNA-1273.815 vaccine
- **Bivalent mRNA-1273.234 vaccine** - Co-formulation of the mRNA-1273.045 vaccine and the mRNA-1273.116 vaccine.

2. PRIMARY PHARMACODYNAMICS

Nonclinical *in vivo* pharmacology studies were conducted in BALB/c mice by the sponsor in support of the development of XBB-containing mRNA vaccines. The submitted studies evaluated the immunogenicity of XBB-containing mRNA vaccines (formulated into a mixture of lipids: SM-102, cholesterol, DSPC, and PEG2000-DMG) given as a primary series, or as a booster dose following primary series vaccination with mRNA-1273. The route of administration of the mRNA vaccines used in the nonclinical *in vivo* pharmacology studies was intramuscular (IM), same as the proposed clinical route.

2.1. IMMUNOGENICITY IN MICE

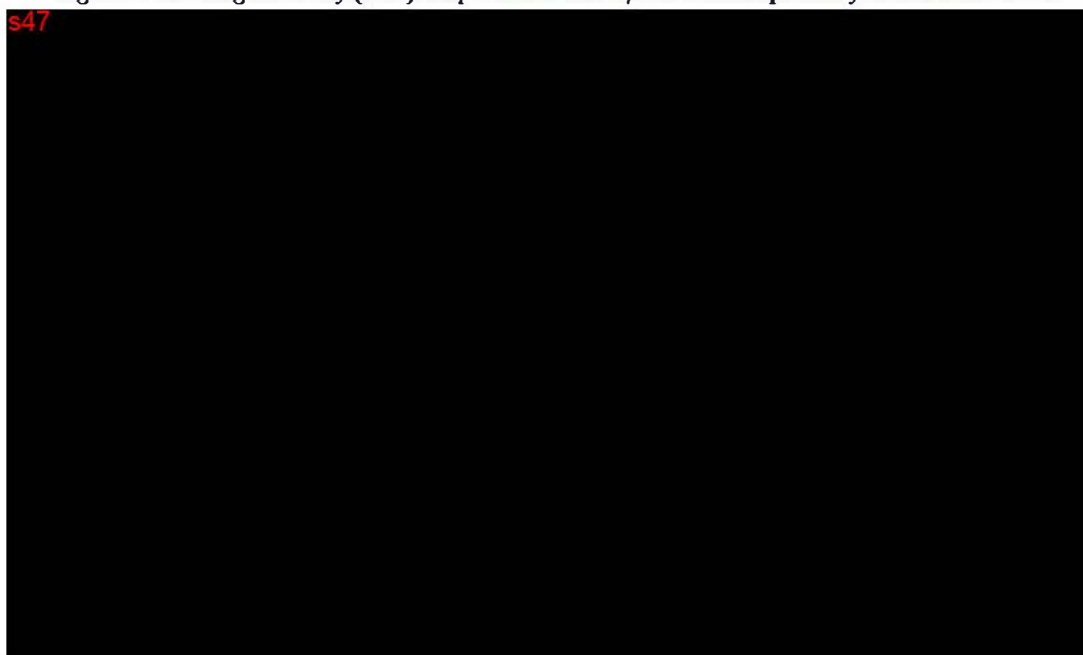
2.1.1. Primary series of monovalent and bivalent SARS-CoV-2 XBB.1.5-containing vaccines

Study details & Major findings						
Study MOD-6037 (09 June 2023)						
Evaluation of immunogenicity of a primary series of monovalent and bivalent SARS-CoV-2 XBB.1.5-containing vaccines in mice						
Test articles & dose formulation	Test Material	mRNA Description	mRNA Lot No(s)	Size (nm)	PDI	EE%
	mRNA-1273.045 ^a	mRNA encoding SARS-CoV-2 BA.4/BA.5 spike protein	DHM-99115	100	0.17	>97
	mRNA-1273.815 ^b	mRNA encoding SARS-CoV-2 XBB.1.5/XBB.1.9.1 spike protein	DH-78330.6	102	0.18	>97
	mRNA-1273.222 ^c	mRNA encoding SARS-CoV-2 spike protein + mRNA encoding SARS-CoV-2 BA.4/BA.5 spike protein (co-formulated 1:1)	DHM-99116	107	0.19	>97
	mRNA-1273.231 ^d	mRNA encoding SARS-CoV-2 BA.4/BA.5 spike protein + mRNA encoding SARS-CoV-2 XBB.1.5/XBB.1.9.1 spike protein (bench side 1:1)	NA	NA	NA	NA
<p>EE%=encapsulation efficiency; LNP=lipid nanoparticle; NA=not applicable; No (s)= numbers; PDI=polydispersity index; S-2P=spike protein with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2=severe acute respiratory syndrome coronavirus 2; SM-102=a custom-manufactured ionizable lipid. Note: All mRNA test material stocks were formulated in SM-102-containing LNPs with a final storage buffer in sodium acetate; sucrose; tris(hydroxymethyl)aminomethane hydrochloride at pH and stored frozen at</p> <p>The EE% is the percentage of mRNA inside the LNP. It is calculated by determining the amount of free mRNA that is accessible to ribogreen in intact LNP and the total mRNA present in detergent-treated LNP; EE%=100 - the percentage of mRNA.</p> <p>^a mRNA-1273.045 vaccine contains a single mRNA encoding the SARS-CoV-2 S-2P antigen of the BA.4/BA.5 subvariants of Omicron. The spike protein of BA.5 is identical to that of BA.4.</p> <p>^b mRNA-1273.815 vaccine contains a single mRNA encoding the SARS-CoV-2 S-2P antigen of the XBB.1.5/XBB.1.9.1 subvariants of Omicron. The spike protein of XBB.1.9.1 is identical to that of XBB.1.5.</p> <p>^c mRNA-1273.222 is a co-formulation of mRNA-1273 and mRNA-1273.045 vaccines.</p> <p>^d mRNA-1273.231 is a 1:1 bench side mix of separately formulated mRNA-1273.045 and mRNA-1273.815 vaccines.</p>						
Test system: Mice (BALB/c, n=8/group)						
Summary of the study design and treatment groups						
Study Design	Group (n=8)	Treatment (IM)	Dose Level (µg)	Dose Schedule	Readouts	
	1	PBS	0	Day 1, Day 22	Serum (Day 21, Day 36) bAb response (ELISA) and Serum (Day 36) nAb response (VSV-PSVNA)	
	2	mRNA-1273.045	1 µg			
	3	mRNA-1273.815	1 µg			
	4	mRNA-1273.222	1 µg			
5	mRNA-1273.231	1 µg				
<p>bAb=binding antibody; ELISA=enzyme linked immunosorbent assay; nAb=neutralising antibody; PBS=phosphate-buffered saline; PSVNA=Pseudovirus neutralisation assay; VSV=vesicular stomatitis virus.</p> <ul style="list-style-type: none"> • Serum samples were analysed for bAb (binding antibody) responses via an enzyme-linked immunosorbent assay (ELISA) • Vesicular Stomatitis Virus-based Pseudovirus Neutralisation Assay (PSVNA) was used for serum nAb (neutralising antibody) responses. 						
Results						
<u>Binding Antibody Responses</u>						
<ul style="list-style-type: none"> • High bAb (IgG) titres against S-2P, were observed following 2-dose primary series with mRNA-1273.045 and mRNA-1273.815 vaccines and bivalent mRNA-1273.222 and mRNA-1273.231 vaccines cf. control (PBS) (shown 						

below in Figure 1).

- On Day 21 (3 weeks after Dose 1), S-2P IgG titres ranged between 2721 – 7991 (presented in pink at the top of figure 1).
- On Day 36 (2 weeks after Dose 2), an increase in the titre by 21- to 39-fold from Day 21 (values ranging from 104627 – 165474) was seen (presented in blue at the top of figure 1).
- XBB.1.5-containing vaccines (monovalent mRNA-1273.815 and bivalent mRNA-1273.231) elicited high S-2P binding antibody titres after the first dose and a substantial increase (27- to 39-fold) after the second dose. The response was comparable to the bAb response observed in mice administered monovalent mRNA-1273.045 (32-fold increase from Day 21 to Day 36) and was higher than the response observed in mice administered bivalent mRNA-1273.222 (21-fold increase from Day 21 to Day 36).

Figure 1. Binding antibody (bAb) responses in BALB/c mice after primary series vaccination



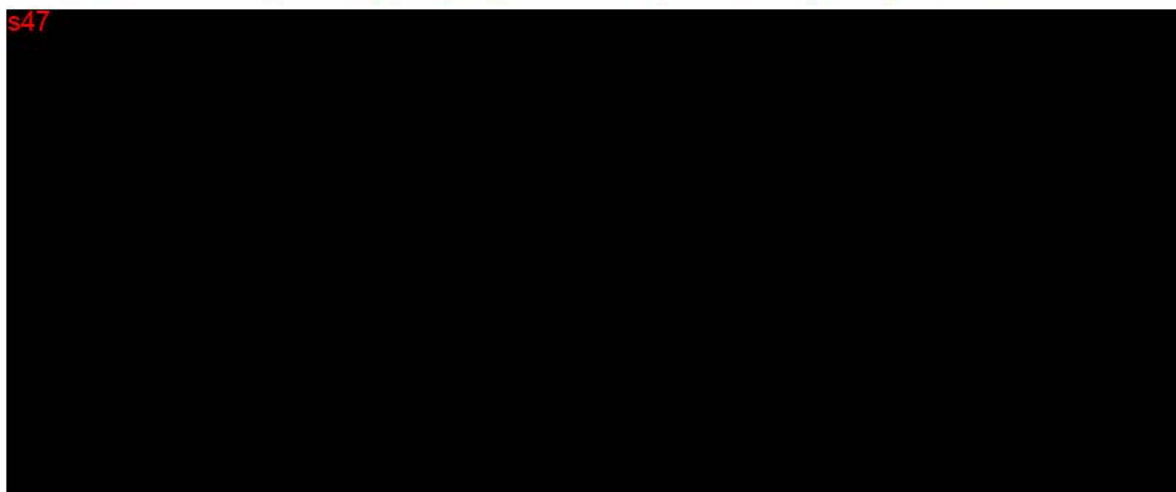
GMT=geometric mean titre; IgG=immunoglobulin G; LOD= limit of detection; PBS=phosphate buffered saline.

Neutralising Antibody Responses

s47

- On Day 36 (2 weeks after the second dose), highest nAb titres (shown in blue on top of figure 2) against XBB.1.5 (16,672 and 18,111) and XBB.1.16 (20,915 and 20,195) were observed in mice receiving the XBB.1.5/XBB.1.9.1 variant-containing vaccines (monovalent mRNA-1273.815 or bivalent mRNA-1273.231, respectively).
- Mice receiving mRNA-1273.045 or mRNA-1273.222 had lower serum nAb titres against XBB.1.5 (937 and 363, respectively) and XBB.1.16 (1269 and 500, respectively).
- nAb titres against XBB.1.5 and XBB.1.16 were comparable.
- Among mice that received XBB.1.5/XBB.1.9.1 variant-containing vaccines (monovalent mRNA-1273.815 or bivalent mRNA-1273.231), nAb titres against D614G were low (61 and 87, respectively).
- mRNA-1273.231 had higher titres (33030) against the BA.4/BA.5 strains *cf.* the mRNA-1273.815 vaccine (2114), consistent with the inclusion of BA.4/BA.5 in the bivalent vaccine.
- In the mRNA-1273.045 group, the order of nAb titres were against BA.4/BA.5 > XBB.1.16 > XBB.1.5 > D614G.
- In the mRNA-1273.222 group, the order of nAb titres were against BA.4/BA.5 > D614G > XBB.1.16 > XBB.1.5.

Figure 2. Neutralising antibody (nAb) responses in BALB/c mice after primary series vaccination



Conclusions

- Following a 2-dose primary series, mRNA-1273.815 and mRNA-1273.231 elicited high bAb and nAb titres against XBB.1.5 and XBB.1.16, indicating strong immunogenicity.
- The nAb titres elicited by mRNA-1273.815 and mRNA-1273.231 against the XBB.1.5 and XBB.1.16 strains were > 4.5-fold higher than those elicited by mRNA-1273.045 or mRNA-1273.222.
- mRNA-1273.815 and mRNA-1273.231 elicited low titres against the SARS-CoV-2 ancestral strains (D614G).
- Titres against XBB.1.5 were comparable to those against XBB.1.16, indicating antigenically similar strains.
- mRNA-1273.231 showed higher titres against BA.4/BA.5 compared to mRNA-1273.815, which was consistent with the inclusion of BA.4/BA.5 in the bivalent vaccine.

Overall, the monovalent mRNA-1273.815 vaccine induces high nAb against Omicron XBB.1.5 and XBB.1.16, but relatively low nAb against the SARS-CoV-2 ancestral strains (D614G).

2.1.2. Monovalent & bivalent SARS-CoV-2 XBB.1.5-containing vaccine boosters

Study details & Major findings						
Study MOD-5827 (02 June 2023)						
Evaluation of immunogenicity of monovalent and bivalent SARS-CoV-2 XBB.1.5-containing vaccine boosters in mice that have received primary series vaccination with mRNA1273.						
Test materials & dose formulation	Test Material	mRNA Description	mRNA Lot No(s)	Size (nm)	PDI	EE%
	mRNA-1273 ^a	mRNA encoding SARS-CoV-2 spike protein	6006920001	110	0.1	83
	mRNA-1273.045 ^b	mRNA encoding SARS-CoV-2 BA.4/BA.5 spike protein	DHM99115	100	0.17	>97
	mRNA-1273.815 ^c	mRNA encoding SARS-CoV-2 XBB.1.5/XBB.1.9.1 spike protein	DH-783306	102	0.18	>97
	mRNA-1273.222 ^d	mRNA encoding SARS-CoV-2 spike protein + mRNA encoding SARS-CoV-2 BA.4/BA.5 spike protein (co-formulated 1:1)	DHM99116	107	0.19	>97
	mRNA-1273.231 ^e	mRNA encoding SARS-CoV-2 BA.4/BA.5 spike protein + mRNA SARS-CoV-2 XBB.1.5 spike protein (bench side 1:1)	NA	NA	NA	NA
EE%=encapsulation efficiency; LNP=lipid nanoparticle; NA=not applicable; No (s)= numbers; PDI=polydispersity index; S-2P=spike protein with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2=severe acute respiratory syndrome coronavirus 2; SM-102=a custom manufactured						

Study Design

ionizable lipid. Note: All mRNA test material stocks were formulated in SM-102-containing LNPs with a final storage buffer in §47 sodium acetate; §47 sucrose; §47 tris(hydroxymethyl)aminomethane hydrochloride at pH §47 and stored frozen at §47. The EE% is the percentage of mRNA inside the LNP. §47
 EE%=100 - §47.
 a mRNA-1273 vaccine contains a single mRNA that encodes the spike protein of the Wuhan-Hu-1 isolate of SARS-CoV-2.
 b mRNA-1273.045 vaccine contains a single mRNA encoding the SARS-CoV-2 S-2P antigen of the BA.4/BA.5 subvariants of Omicron. The spike protein of BA.4 is identical to that of BA.5.
 c mRNA-1273.815 vaccine contains a single mRNA encoding the SARS-CoV-2 S-2P antigen of the XBB.1.5/XBB.1.9.1 subvariants of Omicron. The spike protein of XBB.1.9.1 is identical to that of XBB.1.5.
 d mRNA-1273.222 is a co-formulation of mRNA-1273 and mRNA-1273.045 vaccines.
 e mRNA-1273.231 is a 1:1 bench side mix of separately formulated mRNA-1273.045 and mRNA-1273.815 vaccines.

Test system: Mice (BALB/c, n=8/group)

Summary of the study design and treatment groups

Group (n=8)	Primary Series			Booster			Readouts
	Treatment (IM)	Dose Level (µg)	Dose Schedule	Treatment (IM)	Dose Level (µg)	Dose Schedule	
1	PBS Control	0	Day 1 Day 22	PBS Control	0	Day 92	Serum (Day 21, Day 36, Day 106) bAb response (ELISA)
2	mRNA-1273	0.5		mRNA-1273.045	1		
3	mRNA-1273	0.5		mRNA-1273.815	1		Serum (Day 91, Day 106) nAb response (VSV-PSVNA)
4	mRNA-1273	0.5		mRNA-1273.222	1		
5	mRNA-1273	0.5		mRNA-1273.231	1		

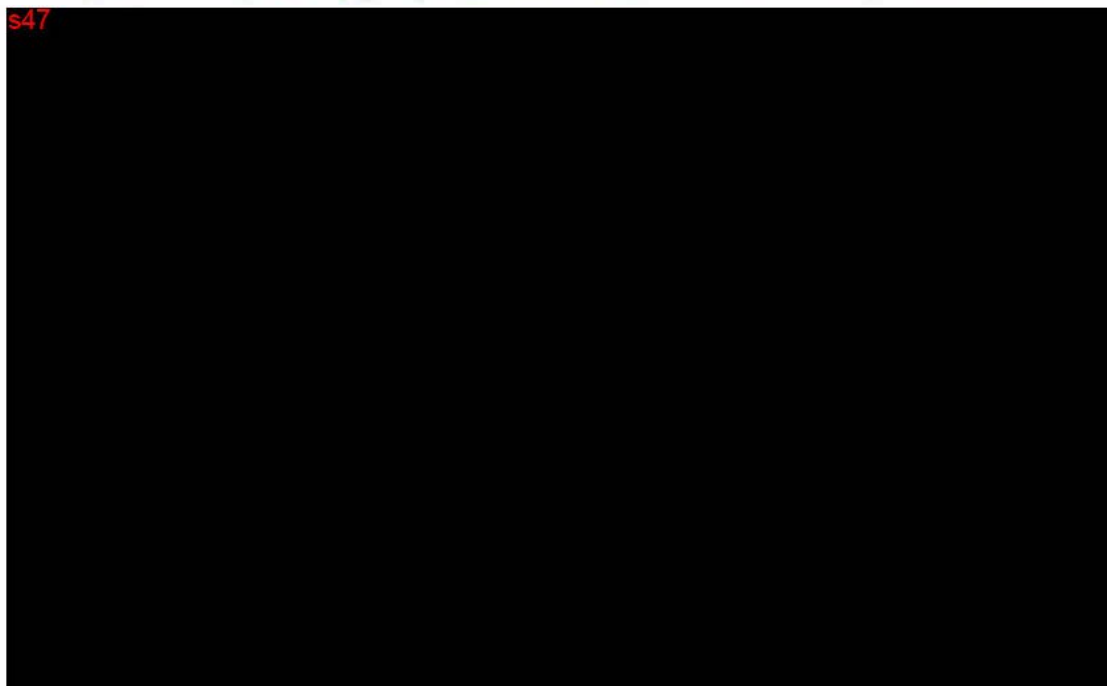
bAb=binding antibody; ELISA=enzyme linked immunosorbent assay; nAb=neutralising antibody; PBS=phosphate-buffered saline; PSVNA=*Pseudovirus* neutralisation assay; VSV=vesicular stomatitis virus.

- Serum samples were analysed for bAb (binding antibody) responses via an enzyme-linked immunosorbent assay (ELISA)
- Vesicular Stomatitis Virus-Based *Pseudovirus* Neutralisation Assay (PSVNA) was used for serum nAb (neutralising antibody) responses.

Results

Binding Antibody Responses

- High bAb (IgG) titres against S-2P, were observed following a 2-dose primary series with mRNA-1273 and boosting (Dose 3) with monovalent mRNA-1273.815, bivalent mRNA-1273.231, monovalent mRNA-1273.045, or bivalent mRNA-1273.222 vaccines compared with control (PBS) (shown below in Figure 3).
- On Day 21 (3 weeks after Dose 1), S-2P IgG titres ranged between 322 – 557 (presented in pink at the top of figure 3).
- On Day 36 (2 weeks after Dose 2), an increase in the titre by 34- to 46-fold from Day 21 (values ranging from 13368 to 20745) was seen (presented in blue at the top of figure 3). Despite all receiving a primary series of mRNA-1273, variability between groups was observed.
- By Day 106 (2 weeks after boosting [Dose 3]) high IgG titres against S-2P (2 to 6-fold increase from Day 36) were observed in all vaccine groups (ranging from 34132 to 85802, presented in green at the top of figure 3).
- mRNA-1273.815 and mRNA-1273.231 elicited comparable S2P-binding IgG titres after boosting that were higher than the bivalent mRNA-1273.222 vaccine.

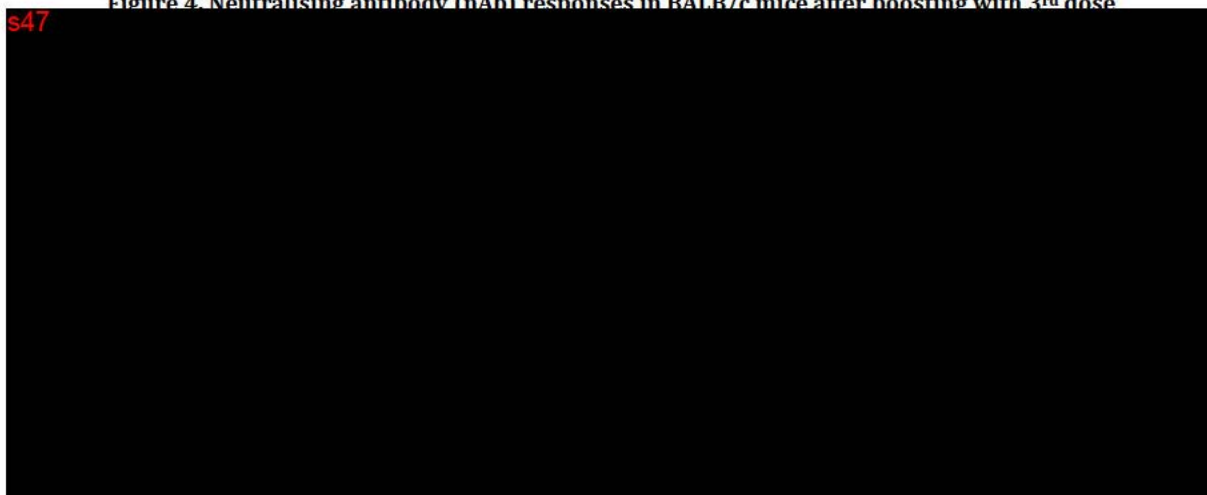
Figure 3. Binding antibody (bAb) responses in BALB/c mice after boosting with the 3rd dose

GMT=geometric mean titre; IgG=immunoglobulin G; LOD= limit of detection; PBS=phosphate buffered saline.

Neutralising Antibody Responses

VSV-based PSVNA

- On Day 91 (pre-boost), high nAb titres against D614G were observed in all vaccine groups (all mice were immunized with 0.5 µg mRNA-1273 vaccine as a primary series). nAb titres against BA.4/BA.5 were lower *cf.* those against D614G. Lowest nAb titres were observed against XBB.1.5 and XBB.1.16.
- On Day 106 (2 weeks after the booster dose), highest nAb titres (shown in purple on top of figure 4) against XBB.1.5 (18 to 26-fold) and XBB.1.16 (14 to 36-fold) from Day 91 to Day 106 were observed in mice that received XBB.1.5/XBB.1.9.1 variant-containing vaccines (monovalent mRNA-1273.815 or bivalent mRNA-1273.231, respectively).
- Moderate increase in serum nAb titres against XBB.1.5 (4.7 to 6.7-fold) and XBB.1.16 (3.8 to 7.2-fold) were seen in mice that receiving mRNA-1273.045 or mRNA-1273.222.
- Mice that received XBB.1.5/XBB.1.9.1 variant-containing vaccines (monovalent mRNA-1273.815 or bivalent mRNA-1273.231), fold increases in serum nAb titres from Day 91 to Day 106 were lowest against D614G (2.1-fold) and BA.4/BA.5 (13-fold) in the mRNA-1273.815 group. However, in the mRNA-1273.231 group, fold increase against these strains were higher (3.8-fold against D614G and 124-fold against BA.4/BA.5).
- In mice that received BA.4/BA.5 variant-containing vaccines (monovalent mRNA-1273.045, bivalent mRNA-1273.222, or bivalent mRNA-1273.231), the highest fold increases in nAb titres were observed against BA.4/BA.5 (94 to 124-fold) followed by titres against D614G (3.8 to 16-fold).

Figure 4. Neutralising antibody (nAb) responses in BALB/c mice after boosting with 3rd dose

Conclusions

- Booster dose of XBB.1.5-containing vaccines (monovalent mRNA-1273.815 or bivalent mRNA-1273.231) elicited high S-2P-bAb (IgG) titres and high nAb response against XBB.1.5 and XBB.1.16 strains.
- Higher nAb response was achieved with mRNA-1273.815, than mRNA-1273.231.
- mRNA-1273.231 had higher titres against the ancestral and BA.4/BA.5 strains *cf.* the mRNA-1273.815 vaccine.

Overall, the booster dose of XBB.1.5-containing vaccines (monovalent mRNA-1273.815 or bivalent mRNA-1273.231) induces high nAb against Omicron XBB.1.5 and XBB.1.16, but relatively low nAb against the SARS-CoV-2 ancestral strains (D614G).

2.1.3. Monovalent & bivalent SARS-CoV-2 XBB.1.16-containing vaccine boosters

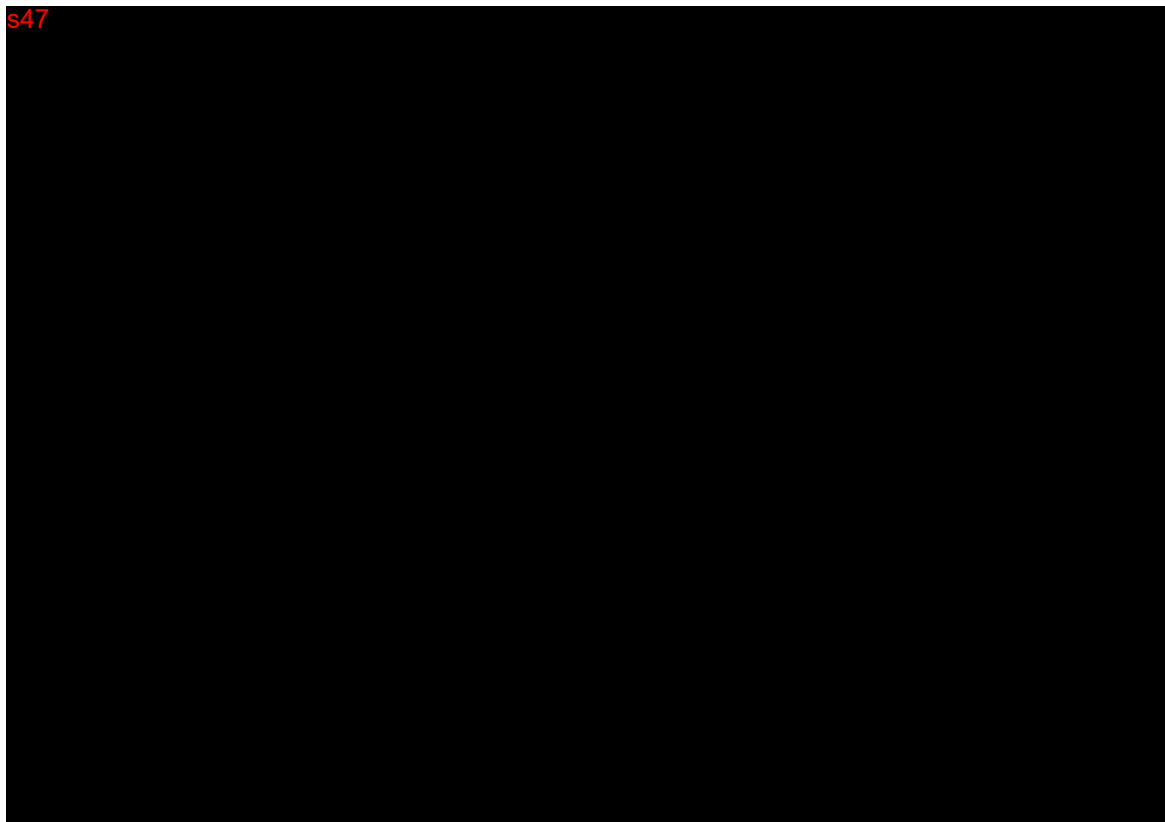
Study details & Major findings																																	
<p>Study MOD-5972 (12 June 2023)</p> <p>Evaluation of immunogenicity of monovalent and bivalent SARS-CoV-2 XBB.1.16-containing vaccine boosters in mice that have received primary series vaccination with mRNA-1273.</p>	<table border="1"> <thead> <tr> <th>Test Material</th> <th>mRNA Description</th> <th>mRNA Lot No(s)</th> <th>Size (nm)</th> <th>PDI</th> <th>EE%</th> </tr> </thead> <tbody> <tr> <td>mRNA-1273^a</td> <td>mRNA encoding SARS-CoV-2 spike protein</td> <td>6006920001</td> <td>110</td> <td>0.1</td> <td>83</td> </tr> <tr> <td>mRNA-1273.116^b</td> <td>mRNA encoding SARS-CoV-2 XBB.1.16 spike protein</td> <td>DH-78330.7</td> <td>107</td> <td>0.16</td> <td>>97</td> </tr> <tr> <td>mRNA-1273.234^c</td> <td>mRNA encoding SARS-CoV-2 BA.4/BA.5 spike protein + mRNA encoding SARS-CoV-2 XBB.1.16 spike protein (bench side 1:1)</td> <td>NA</td> <td>NA</td> <td>NA</td> <td>NA</td> </tr> </tbody> </table> <p>EE%=encapsulation efficiency; LNP=lipid nanoparticle; NA=not applicable; No (s)= numbers; PDI=polydispersity index; S-2P=spike protein with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2=severe acute respiratory syndrome coronavirus 2; SM-102=a custom-manufactured ionizable lipid. Note: All mRNA test material stocks were formulated in SM-102-containing LNPs with a final storage buffer in [redacted] sodium acetate; [redacted] sucrose; [redacted] tris(hydroxymethyl)aminomethane hydrochloride at pH [redacted] and stored frozen at [redacted]. The EE% is the percentage of mRNA inside the LNP. ⁵⁴⁷</p> <p>EE%=100 - ⁵⁴⁷</p> <p>^a mRNA-1273 vaccine contains a single mRNA that encodes the spike protein of the Wuhan-Hu-1 isolate of SARS-CoV-2.</p> <p>^b mRNA-1273.116 vaccine contains a single mRNA encoding the SARS-CoV-2 S-2P antigen of the XBB.1.16 subvariant of Omicron.</p> <p>^c mRNA-1273.234 is a 1:1 bench side mix of separately formulated mRNA-1273.045 (a monovalent vaccine that contains a single mRNA [Lot Number DHM-99115] encoding the SARS-CoV-2 S-2P antigen of the BA.4/BA.5 subvariant of Omicron.; the spike protein of BA.4 is identical to that of BA.5) and mRNA-1273.116 vaccines.</p>	Test Material	mRNA Description	mRNA Lot No(s)	Size (nm)	PDI	EE%	mRNA-1273 ^a	mRNA encoding SARS-CoV-2 spike protein	6006920001	110	0.1	83	mRNA-1273.116 ^b	mRNA encoding SARS-CoV-2 XBB.1.16 spike protein	DH-78330.7	107	0.16	>97	mRNA-1273.234 ^c	mRNA encoding SARS-CoV-2 BA.4/BA.5 spike protein + mRNA encoding SARS-CoV-2 XBB.1.16 spike protein (bench side 1:1)	NA	NA	NA	NA								
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mRNA-1273.116 ^b	mRNA encoding SARS-CoV-2 XBB.1.16 spike protein	DH-78330.7	107	0.16	>97																												
mRNA-1273.234 ^c	mRNA encoding SARS-CoV-2 BA.4/BA.5 spike protein + mRNA encoding SARS-CoV-2 XBB.1.16 spike protein (bench side 1:1)	NA	NA	NA	NA																												
<p>Test materials & dose formulation</p>	<p>Test system: Mice (BALB/c, n=8/group)</p> <p>Summary of the study design and treatment groups</p> <table border="1"> <thead> <tr> <th rowspan="2">Group (n=8)</th> <th colspan="3">Primary Series</th> <th colspan="3">Booster</th> <th rowspan="2">Readouts</th> </tr> <tr> <th>Treatment (IM)</th> <th>Dose Level (µg)</th> <th>Dose Schedule</th> <th>Treatment (IM)</th> <th>Dose Level (µg)</th> <th>Dose Schedule</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>PBS Control</td> <td>0</td> <td rowspan="3">Day 1 Day 22</td> <td>PBS Control</td> <td>0</td> <td rowspan="3">Day 71</td> <td rowspan="3">Serum (Day 21, Day 36, Day 70, Day 85) bAb response (ELISA) Serum (Day 21, Day 70, Day 85) nAb response (VSV-PSVNA)</td> </tr> <tr> <td>2</td> <td>mRNA-1273</td> <td>0.5</td> <td>mRNA-1273.116</td> <td>1</td> </tr> <tr> <td>3</td> <td>mRNA-1273</td> <td>0.5</td> <td>mRNA-1273.234</td> <td>1</td> </tr> </tbody> </table> <p>bAb=binding antibody; ELISA=enzyme linked immunosorbent assay; nAb=neutralising antibody; PBS=phosphate buffered saline; PSVNA=Pseudovirus neutralisation assay; VSV=vesicular stomatitis virus.</p>	Group (n=8)	Primary Series			Booster			Readouts	Treatment (IM)	Dose Level (µg)	Dose Schedule	Treatment (IM)	Dose Level (µg)	Dose Schedule	1	PBS Control	0	Day 1 Day 22	PBS Control	0	Day 71	Serum (Day 21, Day 36, Day 70, Day 85) bAb response (ELISA) Serum (Day 21, Day 70, Day 85) nAb response (VSV-PSVNA)	2	mRNA-1273	0.5	mRNA-1273.116	1	3	mRNA-1273	0.5	mRNA-1273.234	1
Group (n=8)	Primary Series			Booster			Readouts																										
	Treatment (IM)	Dose Level (µg)	Dose Schedule	Treatment (IM)	Dose Level (µg)	Dose Schedule																											
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<p>Study Design</p>																																	

- Serum samples were analysed for bAb (binding antibody) responses *via* an enzyme-linked immunosorbent assay (ELISA)
- Vesicular Stomatitis Virus-Based *Pseudovirus* Neutralisation Assay (PSVNA) was used for serum nAb (neutralising antibody) responses.

Results

Binding Antibody Responses

- bAb (IgG) titres against S-2P were high after a 2-dose primary series (Dose 1 and Dose 2) with mRNA-1273 and boosting (Dose 3) with monovalent mRNA-1273.116 or bivalent mRNA-1273.234, *cf.* control (PBS).
- On Day 21 (3 weeks after Dose 1), S-2P IgG for mRNA-1273.116 and mRNA-1273.234 were 729 and 1914, respectively (presented in pink at the top of figure 5), and by Day 36 (2 weeks after Dose 2), an increase by 17- and 34-fold was observed (to 25004 and 32507, respectively) (presented in blue at the top of figure 5).
- By Day 70 (4 weeks after Dose 2 and pre-booster), S-2P IgG for mRNA-1273.116 and mRNA-1273.234 decreased slightly (20659 and 19532, respectively) (presented in purple at the top of figure 5), which was still a 28- and 10-fold increase, respectively, from Day 21.
- On Day 85 (2 weeks after booster [Dose 3]), mRNA-1273.116 elicited higher bAb antibody titres (79523) *cf.* mRNA-1273.234 (69211) (presented in green at the top of figure 5), both of which were a ~4-fold increase in bAb titres from Day 70 (pre-booster).



GMT=geometric mean titre; IgG=immunoglobulin G; LOD= limit of detection; PBS=phosphate buffered saline.

Neutralising Antibody Responses

VSV-based PSVNA

s47

- On Day 70 (pre-booster), robust nAb titers against D614G were observed in both vaccine groups, as all mice were immunized with 0.5 µg mRNA-1273 vaccine as a primary series.
- nAb titres against BA.4/BA.5 (Day 70) were lower *cf.* to those against D614G.
- Lowest nAb titres were observed against XBB.1.5 and XBB.1.16, indicating substantial immune escape from mRNA-1273 vaccination for these strains.
- From Day 70 to Day 85 (2 weeks after booster [Dose 3]), mice that receiving mRNA-1273.234 demonstrated a higher fold increase in serum nAb titres against XBB.1.5 (25-fold) and XBB.1.16 (33-fold) *cf.* to mice receiving mRNA-1273.116 (XBB.1.5: 17-fold; XBB.1.16: 23-fold).
- The higher nAb response observed post-booster in the mRNA-1273.234 group was considered the study director to be driven by measurable XBB subvariant titres on Day 70 (pre-booster) in 3 of 8 mice in the mRNA-1273.234 group, whereas pre-boost titres against XBB.1.5 or XBB.1.16 for mice in the mRNA-1273.116 group were below the LLOQ.
- Post-booster nAb titre levels against XBB.1.5 and XBB.1.16 were comparable between both treatment groups, indicating that the strains are antigenically similar.
- nAb titres against BA.4/BA.5 were higher in the bivalent mRNA-1273.234 group (30-fold) *cf.* to mRNA-1273.116 (6-fold), which was consistent with the inclusion of BA.4/BA.5 in the vaccine.
- Bivalent mRNA-1273.234 had higher titres against D614G to a greater extent (7.0-fold) compared to the monovalent mRNA-1273.116 (1-fold).

Conclusions

- In pre-booster samples, monovalent mRNA-1273.116 elicited higher S-2P-binding IgG titres than those elicited by bivalent mRNA-1273.234.
- Following a 2-dose primary series with mRNA-1273, animals with booster dose of XBB.1.16-containing vaccines (mRNA-1273.116 or mRNA-1273.234), demonstrated a 4-fold increase in bAb titres.
- Two weeks after the booster, the nAb titres elicited by mRNA-1273.116 and mRNA-1273.234 against the XBB.1.5 and XBB.1.16 strains were 17- to 33-fold higher *cf.* to pre-booster levels.
- mRNA-1273.234 showed higher XBB subvariant nAb responses compared with mRNA-1273.116, which was considered to be driven by measurable XBB subvariant titres observed pre-booster in 3 of 8 mice in the mRNA-1273.234 group.
- nAb titre levels against XBB.1.5 and XBB.1.16 were comparable between both treatment groups following the booster dose.
- nAb titres against BA.4/BA.5 and D614G were higher in the bivalent mRNA-1273.234 group *cf.* to the monovalent mRNA-1273.116 group.

Overall, the booster dose of XBB.1.6-containing vaccines (monovalent mRNA-1273.115 or bivalent mRNA-1273.234) induces high nAb against Omicron BA.4/BA.5, XBB.1.5 and XBB.1.16, compared to the SARS-CoV-2 ancestral strains (D614G).