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**3.2.P.8.3. STABILITY DATA – THERMAL STRESS AND CYCLING – TRIS/SUCROSE DRUG PRODUCT**

Data from stability studies on BNT162b2 Tris/Sucrose drug product lots stored at the thermal stress conditions of  $25 \pm 2$  °C/ $60 \pm 5\%$  RH and  $30 \pm 2$  °C/ $65 \pm 5\%$  RH, and for thermal cycling studies 1, 2, 3, 4 and 5, are presented for three primary drug product lots manufactured by Pfizer, Puurs.

In addition thermal cycling study 6 is presented for one primary drug product lot (EX0490) and 2 drug product lots (FC8273 and FE4394) derived from the initial PPQ campaign at Puurs.

A list of the studies and available data are provided in [Table 3.2.P.8.3-1](#). Results are provided in [Table 3.2.P.8.3-2](#) through [Table 3.2.P.8.3-23](#).

Results are discussed in [Section 3.2.P.8.1 Stability Summary and Conclusions – Tris-Sucrose](#).

**Table 3.2.P.8.3-1. Summary of Tris/Sucrose Drug Product Thermal Stress and Cycling Stability Studies**

Lot Number	Drug Product Batch Use	Stability Start Date	Study Type	Storage Condition	Stability Data Presented	Data Table Location
EX0490 (single dose)	Primary Stability	March 2021	Thermal Stress	25 ± 2 °C/60 ± 5% RH	1 month (Complete)	<a href="#">Table 3.2.P.8.3-2</a>
			Thermal Stress	30 ± 2 °C/65 ± 5% RH	1 month (Complete)	<a href="#">Table 3.2.P.8.3-4</a>
			Thermal Cycling 1: 1 month at -20 ± 5 °C followed by 6 months at 5 ± 3 °C.		4 months (On-going)	<a href="#">Table 3.2.P.8.3-6</a>
			Thermal Cycling 2: 2 months at -20 ± 5 °C followed by 3 months at 5 ± 3 °C.		5 months (Complete) <sup>a</sup>	<a href="#">Table 3.2.P.8.3-9</a>
			Thermal Cycling 3: 3 months at -20 ± 5 °C followed by 3 months at 5 ± 3 °C.		6 months (Complete) <sup>a</sup>	<a href="#">Table 3.2.P.8.3-12</a>
			Thermal Cycling 4: 1 cycle of 1 month at -90 to -60 °C and 1 month at -50 ± 5 °C. 2 cycles of 1 month at -20 ± 5 °C and 1 month at -90 to -60 °C.		6 months (Complete) <sup>a</sup>	<a href="#">Table 3.2.P.8.3-15</a>
			Thermal Cycling 5: 5 cycles of 4 day at -20 ± 5 °C and 1 day at 25 ± 2 °C/60 ± 5% RH. After cycling, move to -50 ± 5 °C until 2 months then transfer to -90 to -60 °C.		6 months (On-going)	<a href="#">Table 3.2.P.8.3-18</a>
EW4564 (multi-dose)	Primary Stability	April 2021	Thermal Cycling 6: 10 months at -90 to -60 °C and 4 months at 5 ± 3 °C		Study Initiated (On-going)	<a href="#">Table 3.2.P.8.3-21</a>
			Thermal Stress	25 ± 2 °C/60 ± 5% RH	1 month (Complete)	<a href="#">Table 3.2.P.8.3-3</a>
			Thermal Stress	30 ± 2 °C/65 ± 5% RH	1 month (Complete)	<a href="#">Table 3.2.P.8.3-5</a>
			Thermal Cycling 1: 1 month at -20 ± 5 °C followed by 6 months at 5 ± 3 °C.		4 months (On-going)	<a href="#">Table 3.2.P.8.3-7</a>
			Thermal Cycling 2: 2 months at -20 ± 5 °C followed by 3 months at 5 ± 3 °C.		5 months (Complete) <sup>a</sup>	<a href="#">Table 3.2.P.8.3-10</a>
			Thermal Cycling 3: 3 months at -20 ± 5 °C followed by 3 months at 5 ± 3 °C.		6 months (Complete) <sup>a</sup>	<a href="#">Table 3.2.P.8.3-13</a>
			Thermal Cycling 4: 1 cycle of 1 month at -90 to -60 °C and 1 month at -50 ± 5 °C. 2 cycles of 1 month at -20 ± 5 °C and 1 month at -90 to -60 °C.		6 months (Complete) <sup>a</sup>	<a href="#">Table 3.2.P.8.3-16</a>

**Table 3.2.P.8.3-1. Summary of Tris/Sucrose Drug Product Thermal Stress and Cycling Stability Studies**

Lot Number	Drug Product Batch Use	Stability Start Date	Study Type	Storage Condition	Stability Data Presented	Data Table Location
			Thermal Cycling 5: 5 cycles of 4 day at $-20 \pm 5$ °C and 1 day at $25 \pm 2$ °C/ $60 \pm 5\%$ RH. After cycling, move to $-50 \pm 5$ °C until 2 months then transfer to $-90$ to $-60$ °C.		6 months (On-going)	<a href="#">Table 3.2.P.8.3-19</a>
EW4565 (multi-dose)	Primary Stability	April 2021	Thermal Cycling: 1 month at $-20 \pm 5$ °C followed by 6 months at $5 \pm 3$ °C.		4 months (On-going)	<a href="#">Table 3.2.P.8.3-8</a>
			Thermal Cycling: 2 months at $-20 \pm 5$ °C followed by 3 months at $5 \pm 3$ °C.		5 months (Complete) <sup>a</sup>	<a href="#">Table 3.2.P.8.3-11</a>
			Thermal Cycling: 3 months at $-20 \pm 5$ °C followed by 3 months at $5 \pm 3$ °C.		6 months (Complete) <sup>a</sup>	<a href="#">Table 3.2.P.8.3-14</a>
			Thermal Cycling 4: 1 cycle of 1 month at $-90$ to $-60$ °C and 1 month at $-50 \pm 5$ °C. 2 cycles of 1 month at $-20 \pm 5$ °C and 1 month at $-90$ to $-60$ °C.		6 months (Complete) <sup>a</sup>	<a href="#">Table 3.2.P.8.3-17</a>
			Thermal Cycling 5: 5 cycles of 4 day at $-20 \pm 5$ °C and 1 day at $25 \pm 2$ °C/ $60 \pm 5\%$ RH. After cycling, move to $-50 \pm 5$ °C until 2 months then transfer to $-90$ to $-60$ °C.		6 months (On-going)	<a href="#">Table 3.2.P.8.3-20</a>
FC8273	Supportive Stability	January 2022	Thermal Cycling 6: 8 months at $-90$ to $-60$ °C and 4 months at $5 \pm 3$ °C		T0 data available <sup>b</sup> (On-going)	<a href="#">Table 3.2.P.8.3-22</a>
FE4394	Supportive Stability	January 2022	Thermal Cycling 6: 7 months at $-90$ to $-60$ °C and 4 months at $5 \pm 3$ °C		T0 data available <sup>b</sup> (On-going)	<a href="#">Table 3.2.P.8.3-23</a>

a. Study terminated early, at indicated timepoint as results demonstrated limited stability at  $-20 \pm 5$  °C. See [Section 3.2.P.8.1 Stability Summary and Conclusion – Tris-Sucrose](#).

b. Only partial data presented for last tested time point.

**Table 3.2.P.8.3-2. Stability Data for Tris/Sucrose SDV Primary Drug Product Lot EX0490  
Stored at  $25 \pm 2$  °C/ $60 \pm 5\%$  RH**

Time	Appearance		pH	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visual)	Visible Particulates		LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content
Acceptance Criteria <sup>a</sup>	White to off-white suspension	May contain white to off-white opaque, amorphous particles					
0	WOS	Meets					
3D	WOS	Meets (EFVP)					
1W	WOS	Meets (EFVP)					
2W	WOS	Meets (EFVP)					
1M	WOS	Meets (EFVP)					

Time	HPLC-CAD				Cell-based Flow Cytometry	Capillary Gel Electrophoresis
	ALC-0315 Content	ALC-0159 Content	DSPC Content	Cholesterol Content	In Vitro Expression	RNA Integrity
Acceptance Criteria <sup>a</sup>						
0						
3D						
1W						
2W						
1M						

a. Acceptance criteria in place at time of testing.

b. Assay was run for information only.

D = Day, W = Week, M = Month, S = To be Scheduled, NS = Not Scheduled at Time Point; EFVP = Essentially free from visible particulates; LNP = Lipid Nanoparticle;

HPLC-CAD = high performance liquid chromatography-charged aerosol detector, WOS = White to off-white suspension



**Table 3.2.P.8.3-3. Stability Data for Tris/Sucrose MDV Primary Drug Product Lot EW4564  
Stored at  $25 \pm 2$  °C/ $60 \pm 5\%$  RH**

Time	Appearance		pH	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visual)	Visible Particulates		LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content
Acceptance Criteria <sup>a</sup>	White to off-white suspension	May contain white to off-white opaque, amorphous particles	4.2 1st. ind.				
0	WOS	Meets					
3D	WOS	Meets (EFVP)					
1W	WOS	Meets (EFVP)					
2W	WOS	Meets (EFVP)					
1M	WOS	Meets (EFVP)					

Time	HPLC-CAD				Cell-based Flow Cytometry	Capillary Gel Electrophoresis
	ALC-0315 Content	ALC-0159 Content	DSPC Content	Cholesterol Content	In Vitro Expression	RNA Integrity
Acceptance Criteria <sup>a</sup>	4.2 1st. ind.					
0						
3D						
1W						
2W						
1M						

a. Acceptance criteria in place at time of testing.

b. Assay run for information only.

D = Day, W = Week, M = Month, S = To be Scheduled, NS = Not Scheduled at Time Point; EFVP = Essentially free from visible particulates; LNP = Lipid Nanoparticle;

HPLC-CAD = high performance liquid chromatography-charged aerosol detector, WOS = White to off-white suspension

**Table 3.2.P.8.3-4. Stability Data for Tris/Sucrose SDV Primary Drug Product Lot EX0490  
Stored at  $30 \pm 2$  °C/ $65 \pm 5\%$  RH**

Time	Appearance		pH	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visual)	Visible Particulates		LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content
Acceptance Criteria <sup>a</sup>	White to off-white suspension	May contain white to off-white opaque, amorphous particles					4.2 1st. ind.
0	WOS	Meets					
3D	WOS	Meets (EFVP)					
1W	WOS	Meets (EFVP)					
2W	WOS	Meets (EFVP)					
1M	WOS	Meets (EFVP)					

Time	HPLC-CAD				Cell-based Flow Cytometry	Capillary Gel Electrophoresis
	ALC-0315 Content	ALC-0159 Content	DSPC Content	Cholesterol Content	In Vitro Expression	RNA Integrity
Acceptance Criteria <sup>a</sup>						4.2 1st. ind.
0						
3D						
1W						
2W						
1M						

a. Acceptance criteria in place at time of testing.

b. Assay run for information only.

D = Day, W = Week, M = Month, S = To be Scheduled, NS = Not Scheduled at Time Point; EFVP = Essentially free from visible particulates; LNP = Lipid Nanoparticle; HPLC-CAD = high performance liquid chromatography-charged aerosol detector, WOS = White to off-white suspension



**Table 3.2.P.8.3-5. Stability Data for Tris/Sucrose MDV Primary Drug Product Lot EW4564  
Stored at  $30 \pm 2$  °C/ $65 \pm 5\%$  RH**

Time	Appearance		pH	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visual)	Visible Particulates		LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content
Acceptance Criteria <sup>a</sup>	White to off-white suspension	May contain white to off-white opaque, amorphous particles	4.2 1st. Ind.				
0	WOS	Meets					
3D	WOS	Meets (EFVP)					
1W	WOS	Meets (EFVP)					
2W	WOS	Meets (EFVP)					
1M	WOS	Meets (EFVP)					

Time	HPLC-CAD				Cell-based Flow Cytometry	Capillary Gel Electrophoresis
	ALC-0315 Content	ALC-0159 Content	DSPC Content	Cholesterol Content	In Vitro Expression	RNA Integrity
Acceptance Criteria <sup>a</sup>						4.2 1st. ind.
0						
3D						
1W						
2W						
1M						

a. Acceptance criteria in place at time of testing.

b. Assay run for information only.

D = Day, W = Week, M = Month, S = To be Scheduled, NS = Not Scheduled at Time Point; EFVP = Essentially free from visible particulates; LNP = Lipid Nanoparticle;

HPLC-CAD = high performance liquid chromatography-charged aerosol detector, WOS = White to off-white suspension

**Table 3.2.P.8.3-6. Thermal Cycling 1 Stability Data for Tris/Sucrose SDV Primary Drug Product Lot EX0490**

Time	Appearance		pH	Subvisible Particles <sup>b</sup>	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visual)	Visible Particulates			LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content
Acceptance Criteria <sup>a</sup>	White to off-white suspension	May contain white to off-white opaque, amorphous particles						
0	WOS	Meets						
1M	WOS	Meets (EFVP)						
Samples pulled for 1M testing								
3M	WOS	Meets (EFVP)						
4M	WOS	Meets (EFVP)						
7M	S	S						

Time	HPLC-CAD				Cell-based Flow Cytometry	Capillary Gel Electrophoresis	Endotoxin (LAL)	Dye Incursion
	ALC-0315 Content	ALC-0159 Content	DSPC Content	Cholesterol Content	In Vitro Expression	RNA Integrity		Container Closure Integrity
Acceptance Criteria <sup>a</sup>								
0								
1M								
Samples pulled for 1M testing								
3M								
4M								
7M								

a. Acceptance criteria in place at time of testing.

b. Subvisible particles are reported per container.

c. Assay run for information only.

W = Week, S = To be Scheduled, EFVP = Essentially free from visible particulates, LNP = Lipid Nanoparticle, HPLC-CAD = high performance liquid chromatography-charged aerosol detector, WOS = White to off-white suspension

**Table 3.2.P.8.3-7. Thermal Cycling 1 Stability Data for Tris/Sucrose MDV Primary Drug Product Lot EW4564**

Time	Appearance		pH	Subvisible Particles <sup>b</sup>	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visual)	Visible Particulates			LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content
Acceptance Criteria <sup>a</sup>	White to off-white suspension	May contain white to off-white opaque, amorphous particles						
0	WOS	Meets						
1M	WOS	Meets (EFVP)						
Samples pulled for 1M test								
3M	WOS	Meets (EFVP)						
4M	WOS	Meets (EFVP)						
7M	S	S						

Time	HPLC-CAD				Cell-based Flow Cytometry In Vitro Expression	Capillary Gel Electrophoresis RNA Integrity	Endotoxin (LAL)	Dye Incursion
	ALC-0315 Content	ALC-0159 Content	DSPC Content	Cholesterol Content				Container Closure Integrity
Acceptance Criteria <sup>a</sup>								
0								
1M								
Samples pulled for								
3M								
4M								
7M								

a. Acceptance criteria in place at time of testing.

b. Subvisible particles are reported per container.

c. Assay run for information only.

W = Week, S = To be Scheduled, EFVP = Essentially free from visible particulates, LNP = Lipid Nanoparticle, HPLC-CAD = high performance liquid chromatography -charged aerosol detector, WOS = White to off-white suspension

**Table 3.2.P.8.3-8. Thermal Cycling 1 Stability Data for Tris/Sucrose MDV Primary Drug Product Lot EW4565**

Time	Appearance		pH	Subvisible Particles <sup>b</sup>	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visual)	Visible Particulates			LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content
Acceptance Criteria <sup>a</sup>	White to off-white suspension	May contain white to off-white opaque, amorphous particles						
0	WOS	Meets						
1M	WOS	Meets (EFVP)						
Samples pulled for 1M testing								
3M	WOS	Meets (EFVP)						
4M	WOS	Meets (EFVP)						
7M	S	S						

Time	HPLC-CAD				Cell-based Flow Cytometry In Vitro Expression	Capillary Gel Electrophoresis RNA Integrity	Endotoxin (LAL)	Dye Incursion
	ALC-0315 Content	ALC-0159 Content	DSPC Content	Cholesterol Content				Container Closure Integrity
Acceptance Criteria <sup>a</sup>								
0								
1M								
3M								
4M								
7M								

a. Acceptance criteria in place at time of testing.

b. Subvisible particles are reported per container.

c. Assay run for information only.

W = Week, S = To be Scheduled, EFVP = Essentially free from visible particulates, LNP = Lipid Nanoparticle, HPLC-CAD = high performance liquid chromatography-charged aerosol detector, WOS = White to off-white suspension

**Table 3.2.P.8.3-9. Thermal Cycling 2 Stability Data for Tris/Sucrose SDV Primary Drug Product Lot EX0490**

Time <sup>a</sup>	Appearance		pH	Subvisible Particles <sup>c</sup>	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visual)	Visible Particulates			LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content
Acceptance Criteria <sup>b</sup>	White to off-white suspension	May contain white to off-white opaque, amorphous particles						
0	WOS	Meets						
1M	WOS	Meets (EFVP)						
2M	WOS	Meets (EFVP)						
Samples pulled for 2M testing.								
4M	WOS	Meets (EFVP)						
5M	WOS	Meets (EFVP)						

Time <sup>a</sup>	HPLC-CAD				Cell-based Flow Cytometry In Vitro Expression	Capillary Gel Electrophoresis RNA Integrity	Endotoxin (LAL)	Dye Incursion
	ALC-0315 Content	ALC-0159 Content	DSPC Content	Cholesterol Content				Container Closure Integrity
Acceptance Criteria <sup>b</sup>								
0								
1M								
2M								
Samples pulled for 2M testing								
4M								
5M								

a. Study terminated at 5 months due to limited stability at  $-20 \pm 5$  °C. See [Section 3.2.P.8.1 Stability Summary and Conclusion – Tris-Sucrose](#).

b. Acceptance criteria in place at time of testing.

c. Subvisible particles are reported per container.

d. Assay run for information only.

W = Week, EFVP = Essentially free from visible particulates, LNP = Lipid Nanoparticle, HPLC-CAD = high performance liquid chromatography-charged aerosol detector, WOS = White to off-white suspension

**Table 3.2.P.8.3-10. Thermal Cycling 2 Stability Data for Tris/Sucrose MDV Primary Drug Product Lot EW4564**

Time <sup>a</sup>	Appearance		pH	Subvisible Particles <sup>c</sup>	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visual)	Visible Particulates			LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content
Acceptance Criteria <sup>b</sup>	White to off-white suspension	May contain white to off-white opaque, amorphous particles						
0	WOS	Meets						
1M	WOS	Meets (EFVP)						
2M	WOS	Meets (EFVP)						
Samples pulled for 2M testing.								
4M	WOS	Meets (EFVP)						
5M	WOS	Meets (EFVP)						

Time <sup>a</sup>	HPLC-CAD				Cell-based Flow Cytometry	Capillary Gel Electrophoresis	Endotoxin (LAL)	Dye Incursion
	ALC-0315 Content	ALC-0159 Content	DSPC Content	Cholesterol Content	In Vitro Expression	RNA Integrity		Container Closure Integrity
Acceptance Criteria <sup>b</sup>								
0								
1M								
2M								
Samples pulled for 2M testing.								
4M								
5M								

a. Study terminated at 5 months due to limited stability at  $-20 \pm 5^\circ\text{C}$ . See [Section 3.2.P.8.1 Stability Summary and Conclusion – Tris-Sucrose](#).

b. Acceptance criteria in place at time of testing.

c. Subvisible particles are reported per container.

d. Assay run for information only.

W = Week, EFVP = Essentially free from visible particulates, LNP = Lipid Nanoparticle, HPLC-CAD = high performance liquid chromatography-charged aerosol detector,

WOS = White to off-white suspension

**Table 3.2.P.8.3-11. Thermal Cycling 2 Stability Data for Tris/Sucrose MDV Primary Drug Product Lot EW4565**

Time <sup>a</sup>	Appearance		pH	Subvisible Particles <sup>c</sup>	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visual)	Visible Particulates			LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content
Acceptance Criteria <sup>b</sup>	White to off-white suspension	May contain white to off-white opaque, amorphous particles						4.2 1st. ind.
0	WOS	Meets						4.2 1st. ind.
1M	WOS	Meets (EFVP)						
2M	WOS	Meets (EFVP)						
Samples pulled for 2M testing. Inventory moved to 5 ± 3 °C for remainder of the study								
4M	WOS	Meets (EFVP)						
5M	WOS	Meets (EFVP)						

Time <sup>a</sup>	HPLC-CAD				Cell-based Flow Cytometry In Vitro Expression	Capillary Gel Electrophoresis RNA Integrity	Endotoxin (LAL)	Dye Incursion Container Closure Integrity
	ALC-0315 Content	ALC-0159 Content	DSPC Content	Cholesterol Content				
Acceptance Criteria <sup>b</sup>								4.2 1st. ind.
Placed @ -20 ± 5 °C (for 2 months)								
0								4.2 1st. ind.
1M								
2M								
Samples pulled for								
4M								4.2 1st. ind.
5M								4.2 1st. ind.

a. Study terminated at 5 months due to limited stability at -20 ± 5 °C. See Section 3.2.P.8.1 Stability Summary and Conclusion – Tris-Sucrose.

b. Acceptance criteria in place at time of testing.

c. Subvisible particles are reported per container.

d. Assay run for information only.

W = Week, EFVP = Essentially free from visible particulates, LNP = Lipid Nanoparticle, HPLC-CAD = high performance liquid chromatography-charged aerosol detector,

WOS = White to off-white suspension



**Table 3.2.P.8.3-12. Thermal Cycling 3 Stability Data for Tris/Sucrose SDV Primary Drug Product Lot EX0490**

Time <sup>a</sup>	Appearance		pH	Subvisible Particles <sup>c</sup>	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visual)	Visible Particulates			LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content
Acceptance Criteria <sup>b</sup>	White to off-white suspension	May contain white to off-white opaque, amorphous particles						
0	WOS	Meets						
1M	WOS	Meets (EFVP)						
2M	WOS	Meets (EFVP)						
3M	WOS	Meets (EFVP)						
Samples pulled for 3M testing.								
5M	WOS	Meets (EFVP)						
6M	WOS	Meets (EFVP)						

  

Time <sup>a</sup>	HPLC-CAD				Cell-based Flow Cytometry In Vitro Expression	Capillary Gel Electrophoresis RNA Integrity	Endotoxin (LAL)	Dye Incursion	
	ALC-0315 Content	ALC-0159 Content	DSPC Content	Cholesterol Content				Container Closure Integrity	
Acceptance Criteria <sup>b</sup>									
0									
1M									
2M									
3M									
Samples pulled for 3M									
5M									
6M									

**Table 3.2.P.8.3-12. Thermal Cycling 3 Stability Data for Tris/Sucrose SDV Primary Drug Product Lot EX0490**

Time <sup>a</sup>	Appearance		pH	Subvisible Particles <sup>c</sup>	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visual)	Visible Particulates			LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content

a. Study terminated at 6 months due to limited stability at  $-20 \pm 5$  °C. See [Section 3.2.P.8.1 Stability Summary and Conclusion – Tris-Sucrose](#).

b. Acceptance criteria in place at time of testing.

c. Subvisible particles are reported per container.

d. Assay run for information only.

W = Week, EFVP = Essentially free from visible particulates, LNP = Lipid Nanoparticle, HPLC-CAD = high performance liquid chromatography-charged aerosol detector, WOS = White to off-white suspension

**Table 3.2.P.8.3-13. Thermal Cycling 3 Stability Data for Tris/Sucrose MDV Primary Drug Product Lot EW4564**

Time <sup>a</sup>	Appearance		pH	Subvisible Particles <sup>c</sup>	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visual)	Visible Particulates			LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content
Acceptance Criteria <sup>b</sup>	White to off-white suspension	May contain white to off-white opaque, amorphous particles						
0	WOS	Meets						
1M	WOS	Meets (EFVP)						
2M	WOS	Meets (EFVP)						
3M	WOS	Meets (EFVP)						
Samples pulled for 3M testing:								
5M	WOS	Meets (EFVP)						
6M	WOS	Meets (EFVP)						

Time <sup>a</sup>	HPLC-CAD				Cell-based Flow Cytometry	Capillary Gel Electrophoresis	Endotoxin (LAL)	Dye Incursion
	ALC-0315 Content	ALC-0159 Content	DSPC Content	Cholesterol Content	In Vitro Expression	RNA Integrity		Container Closure Integrity
Acceptance Criteria <sup>b</sup>								
Placed @ -20 ± 5 °C (for 3 months)								
0								
1M								
2M								
3M								
Samples pulled for 3M testing:								
5M								
6M								

a. Study terminated at 6 months due to limited stability at -20 ± 5 °C. See [Section 3.2.P.8.1 Stability Summary and Conclusion – Tris-Sucrose](#).

b. Acceptance criteria in place at time of testing.

c. Subvisible particles are reported per container.

d. Assay run for information only.

W = Week, EFVP = Essentially free from visible particulates, LNP = Lipid Nanoparticle, HPLC-CAD = high performance liquid chromatography-charged aerosol detector,

WOS = White to off-white suspension

**Table 3.2.P.8.3-14. Thermal Cycling 3 Stability Data for Tris/Sucrose MDV Primary Drug Product Lot EW4565**

Time <sup>a</sup>	Appearance		pH	Subvisible Particles <sup>c</sup>	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visual)	Visible Particulates			LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content
Acceptance Criteria <sup>b</sup>	White to off-white suspension	May contain white to off-white opaque, amorphous particles						
0	WOS	Meets	7.4					
1M	WOS	Meets (EFVP)	7.4					
2M	WOS	Meets (EFVP)	7.3					
3M	WOS	Meets (EFVP)	7.3					
Samples pulled for 3M testing								
5M	WOS	Meets (EFVP)						
6M	WOS	Meets (EFVP)						

  

Time <sup>a</sup>	HPLC-CAD				Cell-based Flow Cytometry	Capillary Gel Electrophoresis	Endotoxin (LAL)	Dye Incursion
	ALC-0315 Content	ALC-0159 Content	DSPC Content	Cholesterol Content	In Vitro Expression	RNA Integrity		Container Closure Integrity
Acceptance Criteria <sup>b</sup>								
0								
1M								
2M								
3M								
Samples pulled for 3M testing								
5M								
6M								

**Table 3.2.P.8.3-14. Thermal Cycling 3 Stability Data for Tris/Sucrose MDV Primary Drug Product Lot EW4565**

Time <sup>a</sup>	Appearance		pH	Subvisible Particles <sup>c</sup>	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visual)	Visible Particulates			LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content

a. Study terminated at 6 months due to limited stability at  $-20 \pm 5$  °C. See [Section 3.2.P.8.1 Stability Summary and Conclusion – Tris-Sucrose](#).

b. Acceptance criteria in place at time of testing.

c. Subvisible particles are reported per container.

d. Assay run for information only.

W = Week, EFVP = Essentially free from visible particulates, LNP = Lipid Nanoparticle, HPLC-CAD = high performance liquid chromatography-charged aerosol detector,

WOS = White to off-white suspension

**Table 3.2.P.8.3-15. Thermal Cycling 4 Stability Data for Tris/Sucrose SDV Primary Drug Product Lot EX0490**

Time <sup>a</sup>	Appearance		pH	Subvisible Particles <sup>c</sup>	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visual)	Visible Particulates			LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content
Acceptance Criteria <sup>b</sup>	White to off-white suspension	May contain white to off-white opaque, amorphous particles						
0	WOS	Meets						
2M	WOS	Meets (EFVP)						
Sample pulled for 2M testing.								
4M	WOS	Meets (EFVP)						
6M	WOS	Meets (EFVP)						

  

Time <sup>a</sup>	HPLC-CAD				Cell-based Flow Cytometry	Capillary Gel Electrophoresis	Dye Incursion
	ALC-0315 Content	ALC-0159 Content	DSPC Content	Cholesterol Content	In Vitro Expression	RNA Integrity	Container Closure Integrity
Acceptance Criteria <sup>b</sup>							
0							
2M							
Sample pulled for 2M testing.							
4M							
6M							

a. Study terminated at 6 months due to limited stability at  $-20 \pm 5$  °C. See [Section 3.2.P.8.1 Stability Summary and Conclusion – Tris-Sucrose](#).

b. Acceptance criteria in place at time of testing.

c. Subvisible particles are reported per container.

d. Assay run for information only.

W = Week, EFVP = Essentially free from visible particulates, LNP = Lipid Nanoparticle, HPLC-CAD = high performance liquid chromatography-charged aerosol detector,

WOS = White to off-white suspension

**Table 3.2.P.8.3-16. Thermal Cycling 4 Stability Data for Tris/Sucrose MDV Primary Drug Product Lot EW4564**

Time <sup>a</sup>	Appearance		pH	Subvisible Particles <sup>c</sup>	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visual)	Visible Particulates			LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content
Acceptance Criteria <sup>b</sup>	White to off-white suspension	May contain white to off-white opaque, amorphous particles						
0	WOS	Meets						
2M	WOS	Meets (EFVP)						
Sample pulled for 2M testing								
4M	WOS	Meets (EFVP)						
6M	WOS	Meets (EFVP)						

Time <sup>a</sup>	HPLC-CAD				Cell-based Flow Cytometry	Capillary Gel Electrophoresis	Dye Incursion
	ALC-0315 Content	ALC-0159 Content	DSPC Content	Cholesterol Content	In Vitro Expression	RNA Integrity	Container Closure Integrity
Acceptance Criteria <sup>b</sup>							
0							
2M							
Sample pulled for 2M testing.							
4M							
6M							

a. Study terminated at 5 months due to limited stability at  $-20 \pm 5^\circ\text{C}$ . See [Section 3.2.P.8.1 Stability Summary and Conclusion – Tris-Sucrose](#).

b. Acceptance criteria in place at time of testing.

c. Subvisible particles are reported per container.

d. Assay run for information only.

W = Week, EFVP = Essentially free from visible particulates, LNP = Lipid Nanoparticle, HPLC-CAD = high performance liquid chromatography-charged aerosol detector,

WOS = White to off-white suspension



**Table 3.2.P.8.3-17. Thermal Cycling 4 Stability Data for Tris/Sucrose MDV Primary Drug Product Lot EW4565**

Time <sup>a</sup>	Appearance		pH	Subvisible Particles <sup>c</sup>	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visual)	Visible Particulates			LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content
Acceptance Criteria <sup>b</sup>	White to off-white suspension	May contain white to off-white opaque, amorphous particles						
0	WOS	Meets		4.2 1st. ind.				
2M	WOS	Meets (EFVP)						
Sample pulled for 2M testing.				4.2 1st. ind.				
4M	WOS	Meets (EFVP)		4.2 1st. ind.				
6M	WOS	Meets (EFVP)						

Time <sup>a</sup>	HPLC-CAD				Cell-based Flow Cytometry	Capillary Gel Electrophoresis	Dye Incursion
	ALC-0315 Content	ALC-0159 Content	DSPC Content	Cholesterol Content	In Vitro Expression	RNA Integrity	Container Closure Integrity
Acceptance Criteria <sup>b</sup>							
0							
2M							
Sample pulled for 2M				4.2 1st. ind.			
4M							
6M							

a. Study terminated at 5 months due to limited stability at  $-20 \pm 5$  °C. See [Section 3.2.P.8.1 Stability Summary and Conclusion – Tris-Sucrose](#).

b. Acceptance criteria in place at time of testing.

c. Subvisible particles are reported per container.

d. Assay run for information only.

W = Week, EFVP = Essentially free from visible particulates, LNP = Lipid Nanoparticle, HPLC-CAD = high performance liquid chromatography-charged aerosol detector,

WOS = White to off-white suspension

**Table 3.2.P.8.3-18. Thermal Cycling 5 Stability Data for Tris/Sucrose MDV Primary Drug Product Lot EX0490**

Time	Appearance		pH	Subvisible Particles <sup>b</sup>	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visual)	Visible Particulates			LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content
Acceptance Criteria <sup>a</sup>	White to off-white suspension	May contain white to off-white opaque, amorphous particles						4.2 1st ind.
0	WOS	Meets						4.2 1st ind.
Cycle 5	WOS	Meets (EFVP)						4.2 1st ind.
Sample pulled for testing after cycle 5. 4.2 1st. ind.								
6M	WOS	Meets (EFVP)						4.2 1st. ind.
12M	S	S						
24M	S	S						

Time	HPLC-CAD				Cell-based Flow Cytometry	Capillary Gel Electrophoresis	Dye Incursion
	ALC-0315 Content	ALC-0159 Content	DSPC Content	Cholesterol Content	In Vitro Expression	RNA Integrity	Container Closure Integrity
Acceptance Criteria <sup>a</sup>							4.2 1st ind.
0							4.2 1st. ind.
Cycle 5							4.2 1st. ind.
Sample pulled for testing after cycle 5. 4.2 1st. ind.							
6M							
12M							
24M							

a. Acceptance criteria in place at time of testing.

b. Subvisible particles are reported per container.

c. Assay run for information only.

W = Week, S = To be Scheduled, EFVP = Essentially free from visible particulates, LNP = Lipid Nanoparticle, HPLC-CAD = high performance liquid chromatography-charged aerosol detector, WOS = White to off-white suspension

**Table 3.2.P.8.3-19. Thermal Cycling 5 Stability Data for Tris/Sucrose MDV Primary Drug Product Lot EW4564**

Time	Appearance		pH	Subvisible Particles b	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visual)	Visible Particulates			LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content
Acceptance Criteria <sup>a</sup>	White to off-white suspension	May contain white to off-white opaque, amorphous particles						
0	WOS	Meets						
Cycle 5	WOS	Meets (EFVP)						
Sample pulled for testing after cycle 5. Inventory transferred to $-50 \pm 5^\circ\text{C}$ until 2 months. Samples transfer to $-90$ to $-60^\circ\text{C}$ for the remainder of the study.								
6M	WOS	Meets (EFVP)						
12M	S	S						
24M	S	S						

Time	HPLC-CAD				Cell-based Flow Cytometry	Capillary Gel Electrophoresis	Dye Incursion
	ALC-0315 Content	ALC-0159 Content	DSPC Content	Cholesterol Content	In Vitro Expression	RNA Integrity	Container Closure Integrity
Acceptance Criteria <sup>a</sup>							
0							
Cycle 5							
Sample pulled for testing after cycle 5. 4.2 1st. ind.							
6M							
12M							
24M							

- a. Acceptance criteria in place at time of testing.  
b. Subvisible particles are reported per container.  
c. Assay run for information only.

W = Week, S = To be Scheduled, EFVP = Essentially free from visible particulates, LNP = Lipid Nanoparticle, HPLC-CAD = high performance liquid chromatography-charged aerosol detector, WOS = White to off-white suspension

**Table 3.2.P.8.3-20. Thermal Cycling 5 Stability Data for Tris/Sucrose MDV Primary Drug Product Lot EW4565**

Time	Appearance		pH	Subvisible Particles <sup>b</sup>	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visual)	Visible Particulates			LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content
Acceptance Criteria <sup>a</sup>	White to off-white suspension	May contain white to off-white opaque, amorphous particles						
0	WOS	Meets						
Cycle 5	WOS	Meets (EFVP)						
Sample pulled for testing after cycle 5								
6M	WOS	Meets (EFVP)						
12M								
24M								

Time	HPLC-CAD				Cell-based Flow Cytometry	Capillary Gel Electrophoresis	Dye Incursion
	ALC-0315 Content	ALC-0159 Content	DSPC Content	Cholesterol Content	In Vitro Expression	RNA Integrity	Container Closure Integrity
Acceptance Criteria <sup>a</sup>							
0							
Cycle 5							
Sample pulled for testing after cycle 5							
6M							
12M							
24M							

a. Acceptance criteria in place at time of testing.

b. Subvisible particles are reported per container.

c. Assay run for information only.

S = To be Scheduled, EFVP = Essentially free from visible particulates, LNP = Lipid Nanoparticle, HPLC-CAD = high performance liquid chromatography-charged aerosol detector, WOS = White to off-white suspension

**Table 3.2.P.8.3-21. Thermal Cycling 6 Stability Data for Tris/Sucrose MDV Primary Drug Product Lot EX0490**

Time	Appearance		pH	Subvisible Particles <sup>b</sup>	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visual)	Visible Particulates			LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content
Acceptance Criteria <sup>a</sup>	White to off-white suspension	May contain white to off-white opaque, amorphous particles						
1M								
3M								
4M								

Time	HPLC-CAD				Cell-based Flow Cytometry	Capillary Gel Electrophoresis	Dye Incursion	Endotoxin (LAL)	Sterility
	ALC-0315 Content	ALC-0159 Content	DSPC Content	Cholesterol Content	In Vitro Expression	RNA Integrity	Container Closure Integrity		
Acceptance Criteria <sup>a</sup>									
1M									
3M									
4M									

a. Acceptance criteria in place at time of testing.

b. Subvisible particles are reported per container.

c. Day 0, samples were pulled for T0 testing. Prior to Day 0, samples were stored at -90 to -60 °C for 10 months

S = To be Scheduled, EFVP = Essentially free from visible particulates, LNP = Lipid Nanoparticle, HPLC-CAD = high performance liquid chromatography-charged aerosol detector, WOS = White to off-white suspension

**Table 3.2.P.8.3-22. Thermal Cycling 6 Stability Data for Tris/Sucrose MDV Process Performance Qualification Drug Product Lot FC8273**

Time	Appearance		pH	Subvisible Particles <sup>b</sup>	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visual)	Visible Particulates			LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content
Acceptance Criteria <sup>a</sup>	White to off-white suspension	May contain white to off-white opaque, amorphous particles						
0°	Meets	meets						
1M								
3M								
4M								

Time	HPLC-CAD				Cell-based Flow Cytometry	Capillary Gel Electrophoresis	Dye Incursion	Endotoxin (LAL)	Sterility
	ALC-0315 Content	ALC-0159 Content	DSPC Content	Cholesterol Content	In Vitro Expression	RNA Integrity	Container Closure Integrity		
Acceptance Criteria <sup>a</sup>									
Inventory Stored at -90 to -60 °C for 8 months									
0 °									
Inventory transferred to 5 °C ± 3 °C for 4 months									
1M									
3M									
4M									

a. Acceptance criteria in place at time of testing.

b. Subvisible particles are reported per container.

c. Day 0, samples were pulled for T0 testing. Prior to Day 0, samples were stored at -90 to -60 °C for 8 months

S = To be Scheduled, NS = Not scheduled, LNP = Lipid Nanoparticle, HPLC-CAD = high performance liquid chromatography-charged aerosol detector, NGD = No growth Detected

**Table 3.2.P.8.3-23. Thermal Cycling 6 Stability Data for Tris/Sucrose MDV Process Performance Qualification Drug Product Lot FE4394**

Time	Appearance		pH	Subvisible Particles <sup>b</sup>	Dynamic Light Scattering (DLS)		Fluorescence Assay	
	Appearance (Visual)	Visible Particulates			LNP Size	LNP Polydispersity	RNA Encapsulation	RNA Content
Acceptance Criteria <sup>a</sup>	White to off-white suspension	May contain white to off-white opaque, amorphous particles						
0 <sup>c</sup>	meets	meets						
1M								
3M								
4M								

Time	HPLC-CAD				Cell-based Flow Cytometry	Capillary Gel Electrophoresis	Dye Incursion	Endotoxin (LAL)	Sterility
	ALC-0315 Content	ALC-0159 Content	DSPC Content	Cholesterol Content	In Vitro Expression	RNA Integrity	Container Closure Integrity		
Acceptance Criteria <sup>a</sup>									
1M									
3M									
4M									

a. Acceptance criteria in place at time of testing.

b. Subvisible particles are reported per container.

c. Day 0, samples were pulled for T0 testing. Prior to Day 0, samples were stored at -90 to -60 °C for 7 months

S = To be Scheduled, NS = Not Scheduled, LNP = Lipid Nanoparticle, HPLC-CAD = high performance liquid chromatography-charged aerosol detector, NGD = No Growth Detected