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3.2.P.8.1. STABILITY SUMMARY AND CONCLUSION – TRIS/SUCROSE DRUG PRODUCT

The commercial shelf life of the BNT162b2 Tris/Sucrose drug product is 12 months when stored at the intended storage condition of -90 to -60 °C. This shelf life is based on 9 months stability data from three BNT162b2 Tris/Sucrose primary drug product lots, 24 weeks Tris/Sucrose development stability data and the current expiry dating established for PBS/Sucrose drug product. The stability data generated for BNT162b2 Tris/Sucrose drug product lots and the Puurs PPQ lots also support the additional storage conditions of 5 ± 3 °C for up to 3 months, within the 12-month shelf-life, supporting the subsequent storage conditions of 2-8 °C for 10 weeks at the point of use.

Drug product lots enrolled in the stability programs are being monitored in accordance with the approved stability protocols. All testing to date has been performed using the analytical methodology and specifications in place at time of testing. The analytical procedures used in the stability programs were developed to monitor the composition, strength, purity, safety and general quality attributes of the drug product.

3.2.P.8.1.1. Drug Product Shelf Life at the Long-Term Storage Temperature of -90 to -60 °C

The shelf life of the BNT162b2 Tris/Sucrose drug product is 12 months when stored at the long-term storage condition of -90 to -60 °C. The shelf life claim is based on 9 months stability data for three BNT162b2 Tris/Sucrose primary drug product lots stored at -90 to -60 °C, 6 months, 6 months and 3 months stability data from the three PPQ lots manufactured at Puurs, 24 weeks Tris/Sucrose development stability data and up to 9 months of stability data for BNT162b2 PBS/Sucrose drug product.

Additionally, the stability data generated to date at 5 ± 3 °C for the BNT162b2 Tris/Sucrose primary drug product lots and the Puurs PPQ lots also support the additional storage condition at 5 ± 3 °C for up to 3 months (within the 12 month shelf life).

3.2.P.8.1.2. In-use Period of Drug Product

Stability data and compatibility studies supporting the in-use period of the BNT162b2 Tris/Sucrose drug product at the administration site is provided in Section 3.2.P.2.6 Compatibility – Tris-Sucrose.

3.2.P.8.1.3. Stability Batches and Studies

The stability program is designed to follow ICH guidelines for stability of drug product (ICH Guideline Q1A: Stability Testing of New Drug Substances and Products; ICH Guideline Q5C: Quality of Biotechnological Products, Stability Testing of Biotechnological/Biological Products). To date, three primary stability lots, seven initial supportive Process Performance Qualification (PPQ) lots filled at 2.25 mL fill volume, three PPQ lots filled at 1.3 mL fill volume and one PPQ lot filled at 0.4 mL volume have been placed on stability. Thereafter, additional lots have been placed on stability. The drug product lots placed on stability were packaged in the glass vials to be used for commercial packaging. A summary of the

Tris/Sucrose drug product lots on stability and current available stability data are shown in [Table 3.2.P.8.1-1](#).

3.2.P.8.1.3.1. Primary Drug Product Lots

Three BNT162b2 Tris/Sucrose primary drug product lots have been placed under long term storage conditions, additional storage conditions and thermal stress and thermal cycling conditions. These primary drug product lots, EX0490, EW4564 and EW4565, were manufactured at Pfizer, Puurs as detailed in Section 3.2.P.2.3 Development History – Tris-Sucrose. The genealogies of these lots are provided in Section 3.2.P.2.3 Lot Genealogy and Usage – Tris-Sucrose.

For each of the three primary drug product lots, the drug substances and LNPs through LNP formation and stabilization were manufactured at [4.2 1st ind.](#) commercial scale using the PBS/Sucrose commercial manufacturing processes, which are unchanged for the Tris/Sucrose formulation. [4.2 1st ind.](#) LNPs were then further processed by buffer exchange into Tris buffer, concentration adjustment, and addition of sucrose. A portion of this Tris/Sucrose bulk drug product was used for fill and finish. The [4.2 1st ind.](#) scale of manufacture for these primary drug product lots was approximately 7% of the planned commercial scale manufacture of [4.2 1st ind.](#) RNA. These primary drug product lots are representative of the proposed commercial Tris/Sucrose drug product lots.

Lot EX0490 was filled at a volume of 0.48 mL per vial to provide a single dose of 30 µg RNA in 0.3 mL injection volume. Lots EW4564 and EW4565 were filled at a volume of 2.25 mL per vial to provide 6 doses of 30 µg RNA in 0.3 mL injection volume. Based upon current understanding of the product characteristics, drug product manufacturing process and ICH Guideline: Q1D Bracketing and Matrix Designs for Stability Testing of New Drug Substance and Products, concepts and principles, all three lots provide support for shelf life establishment of both the single dose and multidose vial.

A summary of the stability data obtained with these three primary drug product lots is provided in [Section 3.2.P.8.1.9](#).

3.2.P.8.1.3.2. Puurs PPQ Lots Filled at 2.25 mL Fill Volume

Ten Puurs PPQ lots are being evaluated for stability under long term and additional storage conditions. These lots were manufactured [4.2 1st ind.](#) and filled into vials at 2.25 mL fill volume, as detailed in [Section 3.2.P.3.5 LNP Formation and Drug Product Formulation and Fill - Tris-Sucrose \[Puurs\]](#) for FC8273, FE4394 and FJ5683, in [Section 3.2.P.3.5. Process Validation and or Evaluation – Manufacturing Process – Filling Line WSL9 \[Puurs\]](#) for FK5132 and [Section 3.2.P.3.5. Process Validation and or Evaluation LNP Production and Drug Product Formulation and Fill Finish – Tris-Sucrose - FB8/WSL5/WSL10/VC2 \[Puurs\]](#) for FP8748, FR5013 and FR7348, in [Section 3.2.P.3.5. Process Validation and or Evaluation - Manufacturing Process – WSL7 \[Puurs\]](#) for FW1374 and in [Section 3.2.P.3.5 Process Validation and/or Evaluation LNP Production and Drug Product Formulation and Fill Finish – Tris-Sucrose – \[4.2 1st ind.\]\(#\) \[Puurs\]](#) for lots GA5554 and FY3701.

An overview of available stability data for all lots for long-term storage at -90 to -60 °C is provided in [Table 3.2.P.8.1-1](#).

3.2.P.8.1.3.3. Puurs PPQ Lots Filled at 1.3 and 0.4 mL Fill Volumes

Three Puurs PPQ lots, one of which was split into two distinct fill lots, are being evaluated for stability under long term and additional storage conditions. These lots, FK5127, FK5128, FK5618, and FM0703 were manufactured at 4.2 1st ind. and filled on WSL10. The first PPQ lot was split 4.2 1st ind. and filled into vials at 1.3 mL, as lot FK5127 and 0.4 mL, as lot FK5128, respectively. The second PPQ lot was filled at 1.3 mL as lot FK5618, as detailed in [Section 3.2.P.3.5 LNP Production Drug Product Formulation and Fill Finish – 1.3 mL and 0.4 mL Fills – Tris-Sucrose \[Puurs\]](#). Three month stability data at the recommended storage condition of -90 to -60 °C are available for these lots, with all results meeting acceptance criteria.

Additionally, 1 month stability data is available for these three lots for storage at -20 ± 5 °C and 3 month stability data at 5 ± 3 °C, with all available results meeting acceptance criteria.

3.2.P.8.1.3.4. Supporting Stability Lots

In addition to the primary stability studies for the Tris/Sucrose drug product discussed above further lots have been included into stability studies as depicted in [Table 3.2.P.8.1-1](#).

Table 3.2.P.8.1-1. Summary of On-going Stability Studies

Lot Number	Stability Study Start	Drug Product Batch Use	Study Type	Storage Condition	Data Available	Study Status
EX0490	March 2021	Primary Stability	Long Term	-90 to -60 °C	9 months	On-going
			Additional	-50 ± 5 °C	1 month	Complete
	Additional		-20 ± 5 °C	6 months	Complete	
	Additional		5 ± 3 °C	6 months	Complete	
	Thermal Stress		25 ± 2 °C/60 ± 5% RH	1 month	Complete	
	Thermal Stress		30 ± 2 °C/65 ± 5% RH	1 month	Complete	
	Thermal Cycling 1		1 month at -20 ± 5 °C followed by 6 months at 5 ± 3 °C.	4 months	On-going	
	Thermal Cycling 2		2 months at -20 ± 5 °C followed by 3 months at 5 ± 3 °C.	5 months	Complete	
	Thermal Cycling 3		3 months at -20 ± 5 °C followed by 3 months at 5 ± 3 °C.	6 months	Complete	
	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	
EW4564	Jan 2022	Primary Stability	Thermal Cycling 5	5 cycles of 4 days 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
			Thermal Cycling 6	10 months at -90 to -60 °C and 4 months at 5±3 °C	Study Initiated	On-going
	April 2021		Long Term	-90 to -60 °C	9 months	On-going
			Additional	-50 ± 5 °C	1 month	Complete
			Additional	-20 ± 5 °C	6 months	Complete
			Additional	5 ± 3 °C	6 months	Complete
			Thermal Stress	25 ± 2 °C/60 ± 5% RH	1 month	Complete
			Thermal Stress	30 ± 2 °C/65 ± 5% RH	1 month	Complete
			Thermal Cycling 1	1 month at -20 ± 5 °C followed by 6 months at 5 ± 3 °C.	4 months	On-going
			Thermal Cycling 2	2 months at -20 ± 5 °C followed by 3 months at 5 ± 3 °C.	5 months	Complete
Thermal Cycling 3	3 months at -20 ± 5 °C followed by 3 months at 5 ± 3 °C.	6 months	Complete			

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3.2.P.8.1. Stability Summary and Conclusion – Tris-Sucrose

Table 3.2.P.8.1-1. Summary of On-going Stability Studies

Lot Number	Stability Study Start	Drug Product Batch Use	Study Type	Storage Condition	Data Available	Study Status
EW4565	April 2021	Primary Stability	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
			Thermal Cycling 5	5 cycles of 4 days 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
			Photostability	With Light Protection and Without Light Protection		Complete
			Long Term	-90 to -60 °C	9 months	On-going
			Additional	-50 ± 5 °C	1 month	Complete
			Additional	-20 ± 5 °C	6 months	Complete
			Additional	5 ± 3 °C	6 months	Complete
			Thermal Cycling 1	1 month at -20 ± 5 °C followed by 6 months at 5 ± 3 °C.	4 months	On-going
			Thermal Cycling 2	2 months at -20 ± 5 °C followed by 3 months at 5 ± 3 °C.	5 months	Complete
			Thermal Cycling 3	3 months at -20 ± 5 °C followed by 3 months at 5 ± 3 °C.	6 months	Complete
FC8273	June 2021	Process Performance Qualification, Puurs FC1	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
			Thermal Cycling 5	5 cycles of 4 days 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
			Long Term	-90 to -60 °C	6 months	On-going
			Additional	-20 ± 5 °C	3 months	Complete

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Table 3.2.P.8.1-1. Summary of On-going Stability Studies

Lot Number	Stability Study Start	Drug Product Batch Use	Study Type	Storage Condition	Data Available	Study Status
FE4394		2.25 mL Fill, 30 µg dose. Supportive Stability	Additional	5 ± 3 °C	6 months ^a	On-going
	Jan 22		Thermal Cycling 6	8 months at -90 to -60 °C and 4 months at 5±3 °C	t0 data available ^a	On-going
	July 2021	Process Performance Qualification, Puurs FC2	Long Term	-90 to -60 °C	6 months ^a	On-going
			Additional	-20 ± 5 °C	1 month	Complete
	Jan 22	2.25 mL Fill, 30 µg dose. Supportive Stability	Additional	5 ± 3 °C	6 months ^a	On-going
FJ5683		2.25 mL Fill, 30 µg dose. Supportive Stability	Thermal Cycling 6	7 months at -90 to -60 °C and 4 months at 5±3 °C	t0 data available ^a	On-going
	Sep 2021	Process Performance Qualification, Puurs FC1	Long Term	-90 to -60 °C	3 months	On-going
			Additional	-20 ± 5 °C	1 month	Complete
		2.25 mL Fill, 30 µg dose. Supportive Stability	Additional	5 ± 3 °C	3 months ^a	On-going
FK5127		Process Performance Qualification, Puurs WSL10	Long Term	-90 to -60 °C	3 months	On-going
	Sep 2021		Additional	-20 ± 5 °C	1 month	Complete
		1.3 mL fill, 10 µg dose. Supportive Stability	Additional	5 ± 3 °C	3 months ^a	On-going
FK5128		Process Performance Qualification, Puurs WSL10	Long Term	-90 to -60 °C	3 months	On-going
	Sep 2021		Additional	-20 ± 5 °C	1 month	Complete
		0.4 mL fill, 3 µg dose. Supportive Stability	Additional	5 ± 3 °C	3 months ^a	On-going
FK5618		Process Performance Qualification, Puurs WSL10	Long Term	-90 to -60 °C	3 months	On-going
	Oct 2021		Additional	-20 ± 5 °C	1 month	Complete
		1.3 mL fill, 10 µg dose. Supportive Stability	Additional	5 ± 3 °C	3 months ^a	On-going
FK5132	Oct 2021	Process Performance	Long Term	-90 to -60 °C	3 months	On-going

Table 3.2.P.8.1-1. Summary of On-going Stability Studies

Lot Number	Stability Study Start	Drug Product Batch Use	Study Type	Storage Condition	Data Available	Study Status
FM0703	Nov 2021	Qualification Puurs WSL9 2.25 mL fill, 30 µg dose. Supportive Stability	Additional	-20 ± 5 °C	1 month	Complete
			Additional	5 ± 3 °C	3 months	On-going
			Long Term	-90 to -60 °C	3 months	On-going
FP8748	Jan 2022	Qualification Puurs WSL9 1.3 mL fill, 10 µg dose. Supportive Stability	Additional	-20 ± 5 °C	1 month	Complete
			Additional	5 ± 3 °C	3 months ^a	On-going
			Long Term	-90 to -60 °C	1 month ^b	On-going
2F1001A	Jan 2022	Process Performance Qualification Puurs WSL5 2.25 mL Fill, 30 µg dose. Supportive Stability	Additional	-20 ± 5 °C	1 month	Complete
			Additional	5 ± 3 °C	1 month	On-going
			Long Term	-90 to -60 °C	2 weeks	On-going
2F1003A	Jan 2022	Process Performance Qualification BNT Marburg / Sanofi 2.25 mL Fill, 30 µg dose.	Additional	-20 ± 5 °C	2 weeks	On-going
			Additional	5 ± 3 °C	2 weeks	On-going
			Long Term	-90 to -60 °C	2 weeks	On-going
FR5013	Feb 2022	Process Performance Qualification Puurs VC2 2.25 mL Fill, 30 µg dose. Supportive Stability	Additional	-20 ± 5 °C	2 weeks	On-going
			Additional	5 ± 3 °C	2 weeks	On-going
			Long Term	-90 to -60 °C	1 month	On-going
FR7348	Feb 2022	Process Performance Qualification Puurs WSL10	Additional	-20 ± 5 °C	1 month	Complete
			Additional	5 ± 3 °C	1 month	On-going
			Long Term	-90 to -60 °C	1 month	On-going
			Additional	-20 ± 5 °C	1 month	Complete

Table 3.2.P.8.1-1. Summary of On-going Stability Studies

Lot Number	Stability Study Start	Drug Product Batch Use	Study Type	Storage Condition	Data Available	Study Status
FW1374	Feb 2022	2.25 mL Fill, 30 µg dose. Supportive Stability	Additional	5 ± 3 °C	1 month ^a	On-going
		Process Performance Qualification Puurs WSL7	Long Term	90 to -60 °C	1 month ^a	On-going
		2.25 mL Fill, 30 µg dose.	Additional	-20 ± 5 °C	1 month ^a	On-going
		Supportive Stability	Additional	5 ± 3 °C	1 month ^a	On-going
2F1004A	Feb 2022	Process Performance Qualification BNT Marburg / Sanofi	Long Term	-90 to -60 °C	2 weeks	On-going
		2.25 mL Fill, 30 µg dose.	Additional	-20 ± 5 °C	2 weeks	On-going
		Supportive Stability	Additional	5 ± 3 °C	2 weeks	On-going
FY3701	Mar 2022	Process Performance Qualification Puurs – 220 g RNA	Long Term	-90 to -60 °C	t0 data available	On-going
		2.25 mL Fill, 30 µg dose.	Additional	-20 ± 5 °C	t0 data available	On-going
		Supportive Stability	Additional	5 ± 3 °C	t0 data available	On-going
GA5554	Apr 2022	Process Performance Qualification Puurs – 70 g RNA	Long Term	-90 to -60 °C	t0 data available ^a	On-going
		2.25 mL Fill, 30 µg dose.	Additional	-20 ± 5 °C	t0 data available ^a	On-going
		Supportive Stability	Additional	5 ± 3 °C	t0 data available ^a	On-going

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

Abbreviations: FC1 = Focus Cell 1 filling line; FC2 = Focus Cell 2 filling line; WSL9 = Washing and Sterilizing Line 9; WSL10 = Washing and Sterilizing Line 10; RH = Relative Humidity

3.2.P.8.1.4. Protocol for Testing at the Long-Term Storage Condition of -90 to -60°C

Vials from primary and supportive PPQ drug product lots were stored at the long-term storage condition of -90 to -60 °C. Testing was performed according to the protocol indicated in Table 3.2.P.8.1-2 for the primary stability lots and [Table 3.2.P.8.1-3](#) and [Table 3.2.P.8.1-4](#) for the supportive PPQ stability lots.

Table 3.2.P.8.1-2. Protocol for BNT162b2 Tris/Sucrose Primary Drug Product Stored at the Long-Term Storage Condition of -90 to -60 °C [Puurs]

Analytical Procedure	Test Interval (Months)
Appearance (Visible)	0, 1, 2, 3, 4, 5, 6, 9, 12, 18, 24
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 3, 6, 12, 18, 24
Container Closure Integrity Test	3, 6, 12, 24
Endotoxin	0, 3, 6, 12, 24
Sterility	0, 12, 24

LNP = Lipid Nanoparticle

Table 3.2.P.8.1-3. Protocol for BNT162b2 Tris/Sucrose Supportive PPQ Drug Product, 2.25 mL fill, Stored at the Long-Term Storage Condition of -90 to -60 °C [Puurs]

Analytical Procedure	Test Interval (Months)
Appearance (Visible)	0, 1 ^a , 3 ^a , 6, 12, 18, 24
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 6, 12, 18, 24
Container Closure Integrity Test	0, 12, 24
Endotoxin	
Sterility	

a. Additional timepoints were added

LNP = Lipid Nanoparticle

Table 3.2.P.8.1-4. Protocol for BNT162b2 Tris/Sucrose PPQ Drug Product, 2.25 mL fill, Stored at the Long-Term Storage Condition of -90 to -60 °C [BioNTech Marburg / Sanofi]

Analytical Procedure	Test Interval
Appearance (Visible)	0, 2W, 1M, 3M, 6M, 9M, 12M, 18M, 24M
Appearance (Visible Particulates)	
Potentiometry (pH)	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Subvisible Particles	
Capillary Gel Electrophoresis (RNA Integrity)	
Cell-based Flow Cytometry (In vitro expression)	
Container Closure Integrity Test	0, 6M, 12M, 24M
Endotoxin	
Sterility	

W = week, M = months, LNP = Lipid Nanoparticle

3.2.P.8.1.5. Protocols for Testing at the Additional Storage Conditions of -50 ± 5 °C, -20 ± 5 °C and 5 ± 3 °C

Vials from the primary drug product lots were stored at the additional storage condition of -50 ± 5 °C and tested per the protocol in [Table 3.2.P.8.1-5](#). Subsequently it was

determined that the -50°C storage condition would not be required and this stability study was stopped at 1 month.

Vials from the primary and supportive PPQ drug product lots were stored at the additional storage conditions of $-20 \pm 5^{\circ}\text{C}$ per the protocols in [Table 3.2.P.8.1-6](#), [Table 3.2.P.8.1-7](#) and [Table 3.2.P.8.1-8](#). It was determined that the -20°C storage condition is not suitable for long term storage therefore, stability protocols for primary stability were stopped at 6 months and supportive PPQ studies were stopped at either 1 or 3 months.

Vials for the primary and supportive PPQ drug product lots were stored at the additional storage condition of $5 \pm 3^{\circ}\text{C}$ and tested per the protocols in [Table 3.2.P.8.1-9](#), [Table 3.2.P.8.1-10](#) and [Table 3.2.P.8.1-11](#).

Table 3.2.P.8.1-5. Protocol for BNT162b2 Tris/Sucrose Drug Product Stored at $-50 \pm 5^\circ\text{C}$

Analytical Procedure	Test Interval (Months)
Appearance (Visible)	0, 1
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	

LNP = Lipid Nanoparticle

Table 3.2.P.8.1-6. Protocol for BNT162b2 Tris/Sucrose Primary Drug Product Stored at $-20 \pm 5^\circ\text{C}$ [Puurs]

Analytical Procedure	Test Interval (Months)
Appearance (Visible)	0, 1, 2, 3, 4, 5, 6
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 3, 6,
Container Closure Integrity Test	3, 6,
Endotoxin	0, 3, 6,

LNP = Lipid Nanoparticle

Table 3.2.P.8.1-7. Protocol for BNT162b2 Tris/Sucrose Supportive PPQ Drug Product Stored at $-20 \pm 5^\circ\text{C}$ [Puurs]

Analytical Procedure	Test Interval (Months)
Appearance (Visible)	0, 1, 3 ^a
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	

a. Three month time point only performed on lot FC8273.

LNP = Lipid Nanoparticle

Table 3.2.P.8.1-8. Protocol for BNT162b2 Tris/Sucrose PPQ Drug Product, 2.25 mL fill, Stored at $-20 \pm 5^\circ\text{C}$ [BioNTech Marburg / Sanofi]

Analytical Procedure	Test Interval
Appearance (Visible)	0, 2W, 1M, 3M
Appearance (Visible Particulates)	
Potentiometry (pH)	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Subvisible Particles	
Capillary Gel Electrophoresis (RNA Integrity)	
Cell-based Flow Cytometry (In vitro expression)	
Container Closure Integrity Test	0, 3M
Endotoxin	
Sterility	

W = week, M = month, LNP = Lipid Nanoparticle

Table 3.2.P.8.1-9. Protocol for BNT162b2 Tris/Sucrose Primary Drug Product Stored at 5 ± 3 °C

Analytical Procedure	Test Interval
Appearance (Visible)	0, 2W, 1M, 3M, 4M, 5M, 6M
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 6M

W = Week, M = Month, LNP = Lipid Nanoparticle

Table 3.2.P.8.1-10. Protocol for BNT162b2 Tris/Sucrose Supportive PPQ Drug Product Stored at 5 ± 3 °C [Puurs]

Analytical Procedure	Test Interval (Months)
Appearance (Visible)	0, 1, 3, 6, 12
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 6, 12
Container Closure Integrity	0, 12
Endotoxin	
Sterility	

LNP = Lipid Nanoparticle

Table 3.2.P.8.1-11. Protocol for BNT162b2 Tris/Sucrose PPQ Drug Product, 2.25 mL fill, Stored at $5 \pm 3^\circ\text{C}$ [BioNTech Marburg / Sanofi]

Analytical Procedure	Test Interval
Appearance (Visible)	0, 2W, 1M, 3M, 6M
Appearance (Visible Particulates)	
Potentiometry (pH)	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Subvisible Particles	
Capillary Gel Electrophoresis (RNA Integrity)	
Cell-based Flow Cytometry (In vitro expression)	
Container Closure Integrity Test	0, 3M, 6M
Endotoxin	
Sterility	

W = week, M = month, LNP = Lipid Nanoparticle

3.2.P.8.1.6. Protocol for Testing at the Thermal Stress Conditions of $25 \pm 2^\circ\text{C}/60 \pm 5\% \text{ RH}$ and $30 \pm 2^\circ\text{C}/60 \pm 5\% \text{ RH}$

To study the effects of thermal stress conditions, two primary drug product lots, EX0490 and EW4564, were stored at $25 \pm 2^\circ\text{C}/60 \pm 5\% \text{ RH}$ and $30 \pm 2^\circ\text{C}/65\% \pm 5 \text{ RH}$ and tested per the protocols indicated in Table 3.2.P.8.1-12 and Table 3.2.P.8.1-13.

Table 3.2.P.8.1-12. Protocol for BNT162b2 Tris/Sucrose Drug Product at the Thermal Stress Condition of $25 \pm 2^\circ\text{C}/60 \pm 5\% \text{ RH}$

Analytical Procedure	Test Interval
Appearance (Visible)	0, 3D, 1W, 2W, 1M
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	

D = Day, W = Week, M = Month, LNP = Lipid Nanoparticle

Table 3.2.P.8.1-13. Protocol for BNT162b2 Tris/Sucrose Drug Product at the Thermal Stress Condition of $30 \pm 2^{\circ}\text{C}/65 \pm 5\% \text{ RH}$

Analytical Procedure	Test Interval
Appearance (Visible)	0, 3D, 1W, 2W, 1M
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	

D = Day, W = Week, M = Month, LNP = Lipid Nanoparticle

3.2.P.8.1.7. Protocol for Thermal Cycling Studies

To study the effects of temporary temperature excursions above the recommended storage temperature and the additional storage temperatures, thermal cycling studies were performed on three primary drug product lots according to the protocols indicated in [Table 3.2.P.8.1-14](#) through [Table 3.2.P.8.1-20](#). Subsequently it was determined that the -20°C storage condition is not suitable for long term storage, therefore the thermal cycling 2, 3 and 4 studies were terminated after the 6 months time point.

To support storage of drug product for 3 months at $5 \pm 3^{\circ}\text{C}$ (including up to 10 weeks at Point of Use), another thermal cycling study is conducted. In this study, three drug product lots are stored at -90 to -60°C for up to 10 months, followed by storage at $2 - 8^{\circ}\text{C}$ for 4 months as indicated in [Table 3.2.P.8.1-19](#).

Table 3.2.P.8.1-14. Protocol for BNT162b2 Tris/Sucrose Drug Product Thermal Cycling 1: $-20 \pm 5^\circ\text{C}$ for 1 month then $2-8^\circ\text{C}$ for 6 months

Thermal Cycling Conditions:	
- Day 0, all inventory placed at $-20 \pm 5^\circ\text{C}$ for 1 month.	
- At 1 month, samples pulled for testing and all other inventory transferred to $5 \pm 3^\circ\text{C}$ for 6 months.	
Analytical Procedure	Test Interval (Months)
Appearance (Visible)	0, 1, 3, 4, 7
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 4, 7
Container Closure Integrity Test	4
Endotoxin	0, 4

LNP = Lipid Nanoparticle

Table 3.2.P.8.1-15. Protocol for BNT162b2 Tris/Sucrose Drug Product Thermal Cycling 2: $-20 \pm 5^\circ\text{C}$ for 2 months then $2-8^\circ\text{C}$ for 3 months

Thermal Cycling Conditions:	
- Day 0, all inventory placed at $-20 \pm 5^\circ\text{C}$ for 2 months.	
- At 2 months, samples pulled for testing and all other inventory transferred to $5 \pm 3^\circ\text{C}$ for 3 months.	
Analytical Procedure	Test Interval (Months)
Appearance (Visible)	0, 1, 2, 4, 5
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 5
Container Closure Integrity Test	5
Endotoxin	0, 5

LNP = Lipid Nanoparticle

Table 3.2.P.8.1-16. Protocol for BNT162b2 Tris/Sucrose Drug Product Thermal Cycling 3: $-20 \pm 5^\circ\text{C}$ for 3 months then $2-8^\circ\text{C}$ for 3 months

Thermal Cycling Conditions: - Day 0, all inventory placed at $-20 \pm 5^\circ\text{C}$ for 3 months. - At 3 months, samples pulled for testing and all other inventory transferred to $5 \pm 3^\circ\text{C}$ for 3 months.	
Analytical Procedure	Test Interval (Months)
Appearance (Visible)	0, 1, 2, 3, 5, 6
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 3, 6
Container Closure Integrity Test	3, 6
Endotoxin	0, 3, 6

LNP = Lipid Nanoparticle

Table 3.2.P.8.1-17. Protocol for BNT162b2 Tris/Sucrose Drug Product Thermal Cycling 4: -90 °C to -60 °C for 1 month then -50 ± 5 °C for 1 month, then 2 cycles of 1 month each at -20 ± 5 °C and -90 °C to -60 °C

Thermal Cycling Conditions: <ul style="list-style-type: none"> - Day 0, all inventory placed at -90 to -60 °C for 1 month. - At 1 month, all inventory transferred to -50 ± 5 °C for 1 month. - At 2 month, samples pulled for testing and all other inventory transferred to -20 ± 5 °C for 1 month. - At 3 month, all inventory transferred to -90 to -60 °C for 1 month. - At 4 month, samples pulled for testing and all other inventory transferred to -20 ± 5 °C for 1 month. - At 5 month, all inventory transferred to -90 to -60 °C for 1 month. - At 6 month, samples pulled for testing. 	
Analytical Procedure	Test Interval (Months)
Appearance (Visible)	0, 2, 4, 6
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 6
LNP = Lipid Nanoparticle	

Table 3.2.P.8.1-18. Protocol for BNT162b2 Tris/Sucrose Drug Product Thermal Cycling 5: 5 cycles at $-20 \pm 5^\circ\text{C}$ for 4 days and $25 \pm 2^\circ\text{C}/60 \pm 5\%$ RH for 1 day, then $-50 \pm 5^\circ\text{C}$ until 2 months and -90 to -60°C

Thermal Cycling Conditions: - 5 cycles each consisting of 4 days at $-20 \pm 5^\circ\text{C}$ move to $25 \pm 2^\circ\text{C}/60 \pm 5\%$ RH for 1 day (25 days total). - Samples pulled for testing after cycle 5 completed. All other inventory moved to $-50 \pm 5^\circ\text{C}$ until 2 months. - At 2 months, all inventory moved to -90 to -60°C for the duration of the study and pulled for testing at 6, 12 and 24 months.	
Analytical Procedure	Test Interval
Appearance (Visible)	0, Cycle 5, 6M, 12M, 24M
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 6, 12, 24
Container Closure Integrity Test	12, 24

M = Months, LNP = Lipid Nanoparticle

Table 3.2.P.8.1-19. Protocol for BNT162b2 Tris/Sucrose Drug Product Thermal Cycling 6: -90 to -60 °C for up to 10 months^a, then 2-8 °C for 4 months

Thermal Cycling Conditions: - Day 0, samples were pulled for T0 testing. Prior to Day 0, samples were stored at -90 to -60 °C for up to 10 months ^a . - All other inventory moved to 5±3 °C for the duration of the study.	
Analytical Procedure	Test Interval (Months)
Appearance (Visible)	0, 1, 3, 4
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	0, 3, 4
Container Closure Integrity Test	0, 4
Endotoxin	0, 4
Sterility	

a. The study is conducted on 3 drug product lots (EX0490, FC8273 and FE4394), which have been held at -90 to -60 °C for the following periods of time (as of the date of manufacture): 10 months (lot EX0490), 8 months (lot FC8273) and 7 months (lot FE4394).

LNP = Lipid Nanoparticle

3.2.P.8.1.8. Protocol for Photostability

To evaluate photostability, one primary drug product lot was exposed to ICH Q1B (option two) light conditions of 1.2 million lux hours of light and 200 watt h/m² of near ultraviolet (UV). Vials were orientated inverted for maximum light exposure in a light chamber set to 5 ± 3 °C, as it is not feasible to maintain the samples at the intended storage condition of -90 to -60 °C for this study and the 5 ± 3 °C condition is considered worst case exposure condition. Testing was performed according to the protocol shown in Table 3.2.P.8.1-20.

Table 3.2.P.8.1-20. Protocol for BNT162b2 Tris/Sucrose Photostability in Drug Product Vials

Analytical Procedure	Test Points
Appearance (Visible)	With Light Protection and Without Light Protection
Appearance (Visible Particulates)	
Potentiometry	
Dynamic Light Scattering (LNP Size)	
Dynamic Light Scattering (LNP Polydispersity)	
Fluorescence Assay (RNA Encapsulation)	
Fluorescence Assay (RNA Content)	
HPLC-CAD (ALC-0315 Content)	
HPLC-CAD (ALC-0159 Content)	
HPLC-CAD (DSPC Content)	
HPLC-CAD (Cholesterol Content)	
Cell-based Flow Cytometry (In vitro expression)	
Capillary Gel Electrophoresis (RNA Integrity)	
Subvisible Particles	

LNP = Lipid Nanoparticle

3.2.P.8.1.9. Summary of Stability Data**3.2.P.8.1.9.1. Summary of Stability Data at the Long-Term Storage Condition of -90 to -60 °C**

The results obtained for BNT162b2 Tris/Sucrose drug product lots stored at the long-term storage condition of -90 to -60 °C are provided in [Section 3.2.P.8.3 Long-Term Storage – Tris-Sucrose](#).

Nine months data are currently available for the three primary drug product lots manufactured by Pfizer, Puurs. All results met acceptance criteria.

Six, six and three months stability data are available for Puurs PPQ lots FC8273, FE4394 and FJ5683 respectively, filled on lines FC1 and FC2 at 2.25 mL. All results available met acceptance criteria.

Three month stability data is available for Puurs PPQ lots FK5127 and FK5618 filled at 1.3 mL and for lot FK5128, filled at 0.4 mL. These 3 lots were filled on the WSL10 line and all results met acceptance criteria.

Partial 3 months stability data are available for Puurs PPQ lots FK5132 and 1 month stability data is available for lot FM0703, for filling at Puurs on WSL9.

For lots FP8748, FR5013, FR7348 and FW1374 filled at 2.25 ml on WSL5, VC2, WSL10 and WSL7 respectively, partial 1 month stability data are available.

All results of testing at the 2-week time point of the BioNTech Marburg/Sanofi PPQ drug product lots 2F1001A, 2F1003A and 2F1004A stored at the long-term storage condition met the acceptance criteria

Data at the initial timepoint is available for lot GA5554 and partial results at 1 month for lot FY3701, supporting the **4.2 1st ind.** lot size range for Tris/Sucrose filled at 2.25 mL.

Overall, the data indicate that there have been no significant changes in terms of quality, purity, or strength for the drug product.

3.2.P.8.1.9.2. Summary of Stability Data at the Additional Storage Conditions of $-50 \pm 5^\circ\text{C}$, $-20 \pm 5^\circ\text{C}$ and $5 \pm 3^\circ\text{C}$

$-50 \pm 5^\circ\text{C}$ Stability

The results obtained for BNT162b2 Tris/Sucrose primary drug product lots stored at $-50 \pm 5^\circ\text{C}$ are provided in [Section 3.2.P.8.3 Additional Storage Conditions – Tris-Sucrose](#). The stability data at study completion at 1 month storage met acceptance criteria.

$-20 \pm 5^\circ\text{C}$ Stability

The results obtained for BNT162b2 Tris/Sucrose drug product lots stored at $-20 \pm 5^\circ\text{C}$ are provided in Section 3.2.P.8.3 Additional Storage Conditions– Tris-Sucrose.

For the three primary drug product lots manufactured at Puurs, all results met acceptance criteria through study completion at 6 months, except for LNP size for lot EW4564. Increasing LNP size was observed for all 3 lots. These results together with those for thermal cycling studies 1, 2 and 3, summarized in [Section 3.2.P.8.1.9.4](#), demonstrate that stability at $-20 \pm 5^\circ\text{C}$ is limited.

For Puurs PPQ lots FC8273, FE4394 and FJ5683 filled on filling lines FC1 and FC2 at 2.25 mL fill volume, stability data at three months, 1 month and 1 month, respectively, met acceptance criteria. This study is complete as it was terminated early as previous results demonstrated limited stability at $-20 \pm 5^\circ\text{C}$.

For Puurs PPQ lots FK5127, FK5128 and FK5618, filled on filling line WSL10 at 1.3 mL, 0.4 mL and 1.3 mL, respectively, stability data at 1 month met acceptance criteria. These studies are complete as they were terminated early as previous results demonstrated limited stability at $-20 \pm 5^\circ\text{C}$.

Stability data at 1 month met acceptance criteria for Puurs PPQ lots FK5132 filled at 2.25 mL and FM0703 filled at 1.3 mL, for filling at Puurs on WSL9. These studies are

complete as they were terminated early as previous results demonstrated limited stability at -20 ± 5 °C.

For Puurs PPQ lots FP8748, FR5013, FR7348 and FW1374 filled at 2.25 mL on filling lines WSL5, VC2, WSL10 and WSL7 respectively, partial one month stability data at -20 °C is available.

All results of testing at the 2-week time point of the BioNTech Marburg/Sanofi PPQ drug product lots 2F1001A, 2F1003A and 2F1004A stored at -20 ± 5 °C met the acceptance criteria.

Data at initial timepoint is available for lot GA5554 and partial results at 1 month for lot FY3701, supporting the **4.2 1st ind.** lot size range for Tris/Sucrose filled at 2.25 mL.

5 ± 3 °C Stability

The results obtained for BNT162b2 Tris/Sucrose drug product lots stored at 5 ± 3 °C are provided in Section 3.2.P.8.3 Additional Storage Conditions – Tris-Sucrose.

For the three primary drug product lots manufactured at Puurs, all data met acceptance criteria through four months storage. Increasing LNP size and polydispersity were observed with OOS results for polydispersity for lots EX0490 and EW4564 at 5 months and for all 3 lots (EX0490, EW4564 and EW4565) at 6 months. Additionally, decreasing In Vitro Expression (IVE) levels were observed with OOS results for lot EX0490 at 6 months.

For Puurs PPQ lots FC8273, FE4394 and FJ5683, filled on filling lines FC1 and FC2 at 2.25 mL fill volume, available stability data at 6 months, 6 months and 3 months, respectively, met acceptance criteria.

For Puurs PPQ lots FK5127 and FK5618, filled on filling line WSL10 at 1.3 mL, and PPQ lot FK5128, filled on filling line WSL10 at 0.4 mL, available stability data at 3 month and met acceptance criteria.

For Puurs PPQ lot FK5132, filled on filling line WSL9 at 2.25 mL, stability data at 3 month met acceptance criteria. For Puurs PPQ lot FM0703, filled on filling line WSL9 at 1.3 mL, stability data at 1 month met acceptance criteria.

For Puurs PPQ lot FP8748, FR5013, FR7348 and FW1374 filled at 2.25 mL on WSL5, VC2, WSL10 and WSL7 respectively, available stability data at 1 month met acceptance criteria.

All results of testing at the 2-week time point of the BioNTech Marburg/Sanofi PPQ drug product lots 2F1001A, 2F1003A and 2F1004A stored at -5 ± 3 °C met the acceptance criteria.

Data at initial timepoint is available for lot GA5554 and partial results at 1 month for lot FY3701, supporting the **4.2 1st ind.** lot size range for Tris/Sucrose filled at 2.25 mL.

These results demonstrate that stability of Tris/Sucrose drug product is limited to approximately 4 months storage at 5 ± 3 °C, supporting 2 weeks storage during internal manufacturing operations and 10 weeks storage at the point of use. These results also demonstrate that these assays detect changes in BNT162b2 drug product indicative of stability.

3.2.P.8.1.9.3. Summary of Stability Data at the Thermal Stress Storage Conditions of 25 ± 2 °C/ $60 \pm 5\%$ RH and 30 ± 2 °C/ $65 \pm 5\%$ RH

To support short term temperature excursions, BNT162b2 Tris/Sucrose primary drug product lots were exposed to the thermal stress conditions of 25 ± 2 °C/ $60 \pm 5\%$ RH and 30 ± 2 °C/ $65 \pm 5\%$ RH. The results obtained are provided in Section 3.2.P.8.3 Thermal Stress and Cycling – Tris-Sucrose.

At 25 ± 2 °C/ $60 \pm 5\%$ RH storage, the stability data at 2 weeks storage met acceptance criteria. At 1 month storage, LNP polydispersity for lot EW4564 and RNA integrity for lots EX0490 and EW4564 did not meet acceptance criteria. Additionally, the IVE level dropped precipitously after 2 weeks to 1 month storage and RNA integrity showed a trend towards lower values.

At 30 ± 2 °C/ $65 \pm 5\%$ RH storage, the stability data at 3 days storage met acceptance criteria. At 1 week storage, IVE expression for lot EX0490 did not meet the acceptance criterion. At 2 weeks storage, RNA integrity for lot EW4564 did not meet the acceptance criterion. At 1 month storage, IVE expression for lot EX0490 and RNA integrity for lots EX0490 and EW4564 did not meet acceptance criteria.

The changes observed in the IVE and RNA integrity results with storage at 25 ± 2 °C/ $60 \pm 5\%$ RH and 30 ± 2 °C/ $60 \pm 5\%$ RH indicate that BNT162b2 Tris/Sucrose drug product has limited stability at these thermal stress conditions.

3.2.P.8.1.9.4. Summary of Stability Data at the Thermal Cycling Storage Conditions

A total of 6 thermal cycling studies are being performed.

Thermal Cycling 1, 2 and 3

The first three cycling studies are evaluating storage at -20 ± 5 °C for 1 month, 2 months and 3 months, respectively, followed by storage at $2-8$ °C for the remainder of the study. This study was set up to support long-term storage and transport at -20 °C followed by short term storage at $2-8$ °C, to facilitate handling and storage at point of use. These studies used the same samples as those for the additional storage condition at -20 ± 5 °C storage for up to 3 months, as detailed in [Section 3.2.P.8.1.5](#), prior to storage at $2-8$ °C.

All three thermal cycling studies consistently showed increasing LNP size and polydispersity and decreasing RNA encapsulation, RNA integrity and IVE levels, including not meeting the acceptance criteria at various timepoints for LNP size and polydispersity and IVE levels. These results demonstrate that stability is limited to a combined 1 month storage at -20 °C followed by 2 months storage at $2-8$ °C.

Based on these results, storage and transport is predominantly restricted to temperatures of -90 to -60 °C, with alternative shipping conditions restricted to ≤ 48 hours at -20 °C and ≤ 80 hours at 2-8 °C, as detailed in [Section 3.2.P.3.3 Fill Finish – Tris-Sucrose – Puurs](#).

Thermal Cycling 4

The results obtained for the BNT Tris/Sucrose primary drug product lots cycled through 1 cycle of -90 to -60 °C for 1 month, then transfer to -50 ± 5 °C for 1 month and 2 cycles of -20 ± 5 °C / -90 to -60 °C for one month each, for a total of six months, are provided in Section 3.2.P.8.3 Thermal Stress and Cycling – Tris-Sucrose. All results met acceptance criteria through study completion.

Thermal Cycling 5

The results obtained for the BNT Tris/Sucrose primary stability lots cycled through 5 cycles of 4 days at -20 ± 5 °C and 1 day at 25 ± 2 °C/ $60 \pm 5\%$ RH followed by storage at -50 ± 5 °C until 2 months and then transferred to -90 to -60 °C for the remainder of the study are provided in Section 3.2.P.8.3 Thermal Stress and Cycling – Tris-Sucrose. All results met acceptance criteria through the five cycles, followed by storage at -50 ± 5 °C until 2 months, followed by storage at -90 to -60 °C for 4 months.

Thermal Cycling 6

This study was initiated to provide additional support for commercial handling conditions of long-term storage at -90 to -60 °C, followed by storage at 2-8 °C. Three lots of drug product stored for up to 10 months at -90 to -60 °C were enrolled in the study and placed at 2-8 °C for testing at 0, 1, 3 and 4 months. Storage at 2-8 °C was initiated in January 2022 and stability data are pending.

3.2.P.8.1.9.5. Summary of Photostability in Drug Product Vials

The results obtained for the BNT Tris/Sucrose primary stability lot EW4564 exposed to ICH Q1B (option two) light conditions are provided in Section 3.2.P.8.3 Stability Data – Photostability – Tris-Sucrose. Results for drug product not protected from light were similar to results for drug product protected from light. A slight decrease in In Vitro Expression and RNA integrity were observed for samples exposed to light as compared to those protected from light, however this is not unexpected, and all results generated met acceptance criteria. The data indicates that drug product does not need to be protected from light.

3.2.P.8.1.9.6. Shelf Life and Conclusions

The stability data obtained to date for the BNT162b2 Tris/Sucrose drug product support 12 months expiry dating when stored at the recommended long-term storage condition of -90 to -60 °C. This shelf life is based on 9 months stability data for the three BNT162b2 Tris / Sucrose primary drug product lots, 6 months and 3 months stability data from three PPQ lots manufactured at Puurs, 24 weeks Tris/Sucrose development stability data and the current 9 months expiry dating established for PBS/Sucrose drug product. Additionally, the stability data generated to date support short term storage at 5 ± 3 °C for up to 3 months, within the 12-month shelf life, supporting the additional storage conditions of 2-8 °C for 10 weeks at the point of use.