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COVID-19 Vaccine (BNT162, PF-07302048)

BB-IND 19736

Response to CBER Comments Received on 20 November 2020 Regarding Overall CMC Information

25 November 2020

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REGARDING THE MANUFACTURE AND TESTING FACILITIES:

QUERY 1

In amendment 119 (submitted on October 21, 2020) Table 1 - EUA and Commercial Supply Chain Manufacturing Nodes, facilities are listed for use in "US and EU markets" (for initial EUA and EUA supply-chain expansion) and "US and/or EU markets" (for commercial/EUA). Please identify the facilities and manufacturing nodes for the production of emergency supply for the US market.

RESPONSE 1

A simplified version of the table provided in amendment 119 (submitted on October 21, 2020) is provided in Table 1 and includes facilities and manufacturing nodes for the production of emergency supply for the US market.

Table 1. Emergency Supply Chain Manufacturing Nodes

<table>
<tr><th colspan="7">Emergency Supply</th></tr>
<tr><td>Drug Substance</td><td colspan="3">Pfizer Andover</td><td colspan="3">BNT Mainz,
and
Rentschler, Germany (Purification)</td></tr>
<tr><td>DS Testing</td><td colspan="3">Pfizer [5]
Andover, [1,2] Chesterfield [2]</td><td colspan="3">BNT Mainz,
BNT IMFS [4],
Rentschler, Germany</td></tr>
<tr><td>LNP, DP</td><td>Polymun</td><td>Pfizer Puurs</td><td>Pfizer Kalamazoo</td><td>Polymun</td><td>DermaPharm</td><td>Pfizer Puurs</td></tr>
<tr><td>Fill/Finish</td><td colspan="2">Pfizer
Puurs
(Lines WSL5, FC2, VC2)</td><td>Pfizer
Kalamazoo
(Lines 8,18)</td><td colspan="3">Pfizer
Puurs
(Line WSL5, FC2, VC2)</td></tr>
<tr><td>DP Release and stability Testing</td><td colspan="2">Pfizer
Andover,[2]
Chesterfield,[2]
Puurs [3]</td><td>Pfizer
Andover,[2]
Chesterfield,[2]
Kalamazoo [3]</td><td colspan="3">Pfizer
Andover,[2]
Chesterfield,[2]
Puurs [3]</td></tr>
</table>

1 Microbial tests: endotoxin, bioburden.
2 Release and Stability testing for Identity, Composition, Strength, Product Purity and/or Process Related Impurities.
3 Microbial tests: endotoxin, sterility. Back-up sterility test sites may be employed.
4 Poly(A) tail and 5'-Cap (Composition) tests may be performed for EUA supplies at Pfizer Andover or Pfizer Chesterfield.
5 Double stranded RNA (Product Related Impurity) Test may be performed for EUA supplies at BNT IMFS.

Literature References

None

SUPPORTING DOCUMENTATION

None

QUERY 2

For each DP manufacturing node, please specify the number of GMP commercial scale DP lots for which data will be available, and submitted for review, at the time of EUA. For DP manufacturing nodes for which GMP commercial scale DP lot data will not be available at the time of initial EUA, please provide an updated time table for submission of these data. Please note that data from at least three GMP commercial-scale DP lots will be required from a DP manufacturing node prior to initial authorization to distribute EUA supplies from that node.

RESPONSE 2

Six lots have been provided to the IND to date as listed in Table 2.

Table 2. Lots Submitted to IND for Emergency Use

Lot	DS Manufacturing Site	LNP Production Site	Fill/Finish Site
EE8492	Pfizer Andover	Polymun Scientific	Pfizer, Puurs
EE8493	Pfizer Andover		
EJ0553	Pfizer Andover		
EJ1685[a]	BioNTech; Rentschler		
EJ1686[a]	BioNTech; Rentschler		
EK1768	Pfizer Andover		

a. IVE data for lots EJ1685 and EJ1686 are provided in 3.2.R BNT162b2 Comparability Report.

Lots manufactured but pending submission to the IND for Emergency Use are provided in Table 3

Table 3. Manufactured Lots Intended for Emergency Supply[a]

Lot	DOM	DS Manufacturing Site	LNP Production Site	Fill/Finish Site	Anticipated CoA Availability
Andover/Kalamazoo/Kalamazoo					
EH9899	7-Oct-2020	Andover	Kalamazoo	Kalamazoo (Line 8)	30-Nov-2020
EK5730	22-Oct-2020	Andover	Kalamazoo	Kalamazoo (Line 8)	30-Nov-2020
EK9231	04-Nov-2020	Andover	Kalamazoo	Kalamazoo (Line 18)	30-Nov-2020
EL1283	11-Nov-2020	Andover	Kalamazoo	Kalamazoo (Line 18)	14-Dec-2020
EL1284	17-Nov-2020	Andover	Kalamazoo	Kalamazoo (Line 18)	15-Dec-2020
EL3246	19-Nov-2020	Andover	Kalamazoo	Kalamazoo (Line 8)	29-Dec-2020
BioNTech;Rentschler/Polymun/Puurs					
EL0141	29-Oct-2020	BNT;RNT	Polymun[b]	Puurs (WSL5)	7-Dec-2020
EK4241	12-Nov-2020	BNT;RNT	Polymun[b]	Puurs (WSL5)	15-Dec-2020

Table 3. Manufactured Lots Intended for Emergency Supply[a]

Lot	DOM	DS Manufacturing Site	LNP Production Site	Fill/Finish Site	Anticipated CoA Availability
BioNTech;Rentschler/DermaPharm/Puurs					
EL0140	29-Oct-2020	BNT;RNT	DermaPharm	Puurs (WSL5)	7-Dec-2020
EL0142	29-Oct-2020	BNT;RNT	DermaPharm	Puurs (WSL5)	7-Dec-2020
EK4237	5-Nov-2020	BNT;RNT	DermaPharm	Puurs (WSL5)	7-Dec-2020
EK4243	5–Nov-2020	BNT;RNT	DermaPharm	Puurs (WSL5)	7-Dec-2020
EK4244	5-Nov-2020	BNT;RNT	DermaPharm	Puurs (WSL5)	14-Dec-2020
EK4245	12-Nov-2020	BNT;RNT	DermaPharm	Puurs (WSL5)	16-Dec-2020
BioNTech;Rentschler/Puurs/Puurs					
EL0725	30-Oct-2020	BNT;RNT	Puurs	Puurs (FC2)	7-Dec-2020
EL0739	03-Nov-2020	BNT;RNT	Puurs	Puurs (FC2)	7-Dec-2020
EL1484	04-Nov-2020	BNT;RNT	Puurs	Puurs (FC2)	7-Dec-2020
EJ6795	12-Nov-2020	Andover	Puurs	Puurs (FC2)	14-Dec-2020

a: All lots are currently pending release.
b: Data from 6 GMP commercial-scale Polymun DP lots have already been submitted, thus complying with the 3 lot requirement to distribute EUA supplies from that node.
Abbreviations: BNT = BioNTech; RNT = Rentschler
Note: Final lot release by the Quality Unit occurs after CoA availability.

Literature References

None

SUPPORTING DOCUMENTATION

New or Replaced Supporting Documentation

3.2.R BNT162b2 Comparability Report, Replaced

Previously submitted supporting documentation

None

QUERY 3

Please note that data from all PPQ studies at all manufacturing nodes must be completed prior to submission of a BLA.

RESPONSE 3

The sponsor acknowledges that data from all PPQ studies at all manufacturing nodes must be completed prior to submission of a BLA.

Literature References

None

SUPPORTING DOCUMENTATION

None

QUERY 4

In the absence of adequate PPQ data for drug product manufactured at multiple sites, it will be necessary for you to submit final COAs for lots to be distributed under EUA at least 48 hours prior to lot distribution. Please submit a plan and schedule for lot distribution under EUA.

RESPONSE 4

Pfizer plans to submit batches of available Certificates of Analyses (CoAs) twice a week (e.g. on Mondays and Thursdays). This schedule may be increased prior to December 11 to facilitate release of initial supplies and in accordance with the requirements laid out in Question 2. Lots will continue to be manufactured in support of emergency supply and submitted to the IND at least 48 hours prior to lot distribution.

Table 4. Anticipated Distribution Dates

Lot	DOM	DS Manufacturing Site	LNP Production Site	Fill/Finish Site	Anticipated CoA Availability	Anticipated Distribution Date
Andover/Kalamazoo/Kalamazoo						
EH9899	7-Oct-2020	Andover	Kalamazoo	Kalamazoo (Line 8)	30-Nov-2020	Authorization Date
EK5730	22-Oct-2020	Andover	Kalamazoo	Kalamazoo (Line 8)	30-Nov-2020	Authorization Date
EK9231	04-Nov-2020	Andover	Kalamazoo	Kalamazoo (Line 18)	30-Nov-2020	Authorization Date
EL1283	11-Nov-2020	Andover	Kalamazoo	Kalamazoo (Line 18)	14-Dec-2020	Dec 16-2020
EL1284	17-Nov-2020	Andover	Kalamazoo	Kalamazoo (Line 18)	15-Dec-2020	Dec 19-2020
EL3246	19-Nov-2020	Andover	Kalamazoo	Kalamazoo (Line 8)	29-Dec-2020	Dec 31-2020
Andover/Polymun/Puurs						
EE8492	05-Aug-2020	Andover	Polymun	Puurs (WSL5)	Data provided	N/A[a]
EE8493	05-Aug-2020	Andover	Polymun	Puurs (WSL5)	Data provided	N/A[a]
EJ0553	25-Sep-2020	Andover	Polymun	Puurs (WSL5)	Data provided	Authorization Date
EK1768	16-Oct 2020	Andover	Polymun	Puurs (WSL5)	IVE Results pending	Authorization Date
BioNTech;Rentschler/Polymun/Puurs						
EJ1685	05-Oct-2020	BNT;RNT	Polymun	Puurs (WSL5)	Data provided	Authorization Date
EJ1686	07-Oct-2020	BNT;RNT	Polymun	Puurs (WSL5)	Data provided	Authorization Date
EL0141	29-Oct-2020	BNT;RNT	Polymun	Puurs (WSL5)	7-Dec-2020	Authorization Date
EK4241	12-Nov-2020	BNT;RNT	Polymun	Puurs (WSL5)	15-Dec-2020	Dec 19-2020

Table 4. Anticipated Distribution Dates

Lot	DOM	DS Manufacturing Site	LNP Production Site	Fill/Finish Site	Anticipated CoA Availability	Anticipated Distribution Date
BioNTech;Rentschler/DermaPharm/Puurs						
EL0140	29-Oct-2020	BNT;RNT	DermaPharm	Puurs (WSL5)	7-Dec-2020	Authorization Date
EL0142	29-Oct-2020	BNT;RNT	DermaPharm	Puurs (WSL5)	7-Dec-2020	Authorization Date
EK4237	5-Nov-2020	BNT;RNT	DermaPharm	Puurs (WSL5)	7-Dec-2020	Authorization Date
EK4244	5-Nov-2020	BNT;RNT	DermaPharm	Puurs (WSL5)	7-Dec-2020	Authorization Date
EK4243	5-Nov-2020	BNT;RNT	DermaPharm	Puurs (WSL5)	14-Dec-2020	20-Dec-2020
EK4245	12-Nov-2020	BNT;RNT	DermaPharm	Puurs (WSL5)	16-Dec-2020	30-Dec-2020
BioNTech;Rentschler/Puurs/Puurs						
EL0725	30-Oct-2020	BNT;RNT	Puurs	Puurs (FC2)	7-Dec-2020	Authorization Date
EL0739	03-Nov-2020	BNT;RNT	Puurs	Puurs (FC2)	7-Dec-2020	Authorization Date
EL1484	04-Nov-2020	BNT;RNT	Puurs	Puurs (FC2)	7-Dec-2020	Authorization Date
EJ6795	12-Nov-2020	Andover	Puurs	Puurs (FC2)	14-Dec-2020	30-Dec-2020

a. Not intended for distribution under Emergency Use in the United States due to differences in applied label
Abbreviations: BNT = BioNTech; RNT = Rentschler

Literature References

None

SUPPORTING DOCUMENTATION

None

QUERY 5

For lots with observable LMS, please describe the percent range of the LMS peak area detected by CGE.

AND

QUERY 6

Please state the percentage of DP lots that have the LMS peak and provide a list of all impacted DP lots, including information on the DP manufacturing site as well as the associated DS lots and lipid lots/sources used for DP manufacture.

RESPONSE to 5 and 6

Table 5 is a list of drug product lots with results from capillary gel electrophoresis (CGE) including the percent late migrating species. The RNA integrity assay reports the % time-corrected area of the main peak, with all other peaks (RNA fragments preceding main peak and LMS RNA species trailing main peak) influencing the reported % RNA integrity value. Most lots (14 out of 20) have some level of late migrating species reported.

The release specification for RNA integrity controls both RNA fragments and LMS since both of these species lead to lower RNA integrity. The release specification limit has been tightened to ≥55% to ensure the integrity of RNA is maintained through the point of use. For lots that meet release specification acceptance criteria, LMS ranged up to 16%. A representative electropherogram of a recent lot with 9% late migrating species is presented in Figure 1.

Figure 1. CGE Electropherogram of DP Lot EJ1685

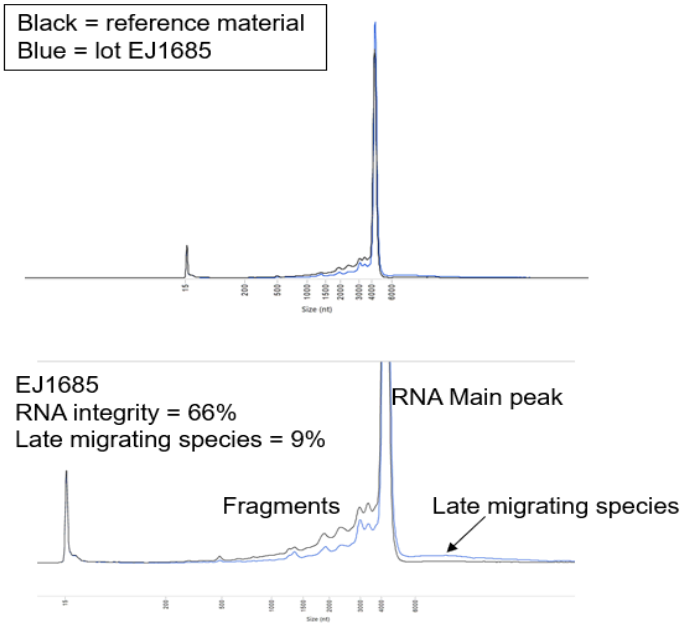


Table 5. Lot Genealogy with RNA Integrity and LMS Levels

LNP Site	DP Lot #	F/F Site	LMS	RNA Integrity	ALC-0315 Mfr	ALC-0315 Lot #	ALC-0159 Mfr	ALC-0159 Lot #	DSPC Mfr	DSPC Lot #	Cholesterol Mfr	Cholesterol Lot #
PLY	EE8492	Puurs	< QL[c]	55%	Avanti	GALC03 15-12	Avanti	GALC01 59-12	Lipoid	556500-219039 5-01	Wilshire[b]	P60349
PLY	EE8493	Puurs	< QL[c]	55%	Avanti	GALC03 15-13	Avanti	GALC01 59-12	Lipoid	556500-219039 5-01	Wilshire[b]	P90390
KZO	EJ0701	KZO	17%	52%[a]	Croda	DTP/465 /1	Avanti	ALC015 9-105	Avanti	DSPCII S-112	Avanti	SCHOLB-105
DMP	EJ0724	Puurs	< QL[c]	71%	Avanti	GALC03 15-14	Avanti	ALC015 9-105	Avanti	DSPCII S-111	Avanti	SCHOLB-105
PLY	EJ0553	Puurs	< QL[c]	68%	Avanti	GALC03 15-12/ GALC03 15-13	Avanti	GALC01 59-12	Lipoid	556500-219039 5-01	Wilshire[b]	P90390
KZO	EH9899	KZO	< QL[c]	59%	Croda	DTP/465 /3	Avanti	ALC015 9-105	Avanti	DSPCII S-112	Avanti	SCHOLB-105
PLY	EK1768	Puurs	< QL[c]	60%	Croda	1755889	Avanti	ALC015 9-104	Lipoid	556500-220042 1-01	Wilshire[b]	P90390
PLY	EJ1685	Puurs	9%	66%	Croda	DTP/465 /3	Avanti	GALC01 59-12	Lipoid	556500-2180372 -01 556500-2200421 -01	Wilshire[b]	P90390
PLY	EJ1686	Puurs	6%	69%	Croda	DTP/465 /3	Avanti	GALC01 59-12/ ALC015 9-104	Lipoid	556500-2200421 -01	Wilshire[b]	P90390
DMP	EJ1688	Puurs	10%	63%	Croda	1755889	Avanti	ALC015 9-105	Avanti	DSPCII S-111	Avanti	SCHOLB-105
PLY	EK4176	Puurs	10%	65%	Croda	1760275	Avanti	ALC015 9-104	Lipoid	556500-220042	Wilshire[b]	P90390

Table 5. Lot Genealogy with RNA Integrity and LMS Levels

LNP Site	DP Lot #	F/F Site	LMS	RNA Integrity	ALC-0315 Mfr	ALC-0315 Lot #	ALC-0159 Mfr	ALC-0159 Lot #	DSPC Mfr	DSPC Lot #	Cholesterol Mfr	Cholesterol Lot #
DMP	EK4175	Puurs	16%	58%	Croda	1760275	Avanti	ALC0159-106	Avanti	1-01 DSPCII S-111	Avanti	SCHOLB-105
DMP	EJ1691	Puurs	24%	51%[a]	Croda	1760275	Avanti	ALC0159-106	Avanti	DSPCII S-111	Avanti	SCHOLB-105
KZO	EK5730	KZO	10%	62%	Croda	DTP/465/3	Avanti	ALC0159-105	Avanti	DSPCII S-112	Avanti	SCHOLB-105
DMP	EL0140	Puurs	6%	69%	Croda	1755889	Avanti	ALC0159-106	Avanti	DSPCII S-111	Avanti	SCHOLS-129
PLY	EL0142	Puurs	6%	69%	Croda	1755889	Avanti	ALC0159-106	Avanti	DSPCII S-111	Avanti	SCHOLS-129
PLY	EL0141	Puurs	5%	67%	Croda	1755889	Avanti	ALC0159-104	Lipoid	556500-220042 1-01	Wilshire[b]	P90390
Puurs	EL0725	Puurs	9%	63%	Croda	DTP/465/3	Avanti	ALC0159-106	Avanti	DSPCII S-112	Avanti	SCHOLS-129
DMP	EK4237	Puurs	9%	64%	Croda	1755889 and 1760275	Avanti	ALC0159-107	Avanti	DSPCII S-111	Avanti	SCHOLS-129
Puurs	EL0739	Puurs	6%	67%	Croda	DTP/465/3	Avanti	ALC0159-106	Avanti	DSPCII S-112	Avanti	SCHOLB-105

a. does not meet the tightened DP RNA Integrity release specification, not intended for emergency supply
b. Wilshire is now Evonik.
c. QL=quantitation limit (3%).

QL= quantitation limit, DMP = Dermapharm, PLY = Polymun, KZO = Kalamazoo, LMS = late migrating species, LNP = lipid nanoparticle, F/F = fill/finish

QUERY 7

Your investigation regarding the identity of the LMS is based on the evaluation of one DP engineering lot (EG5411). Please provide similar data for other impacted DP lots. In addition, please provide data to support that the presence of LMS peak will have no impact on the stability profile of DP.

RESPONSE 7

Characterization of additional DP lots are summarized below.

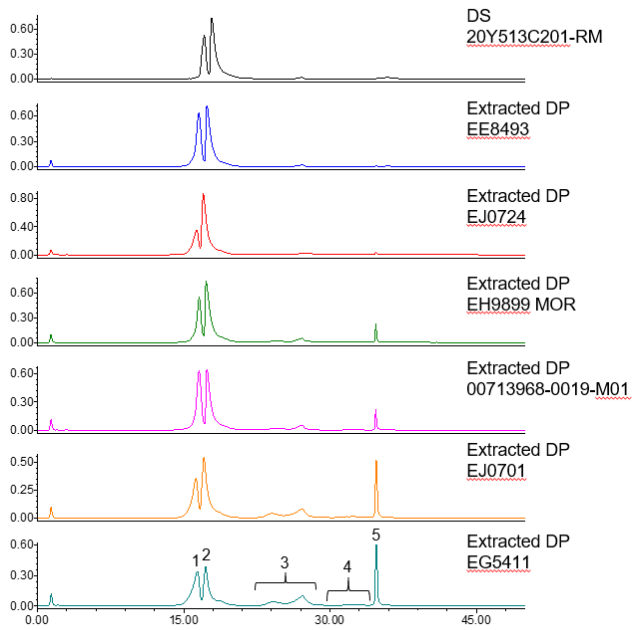
Table 6. Characterization of LMS in DP Lots in addition to EG5411

Characterization Method	Lots (release LMS result)	Conclusion
IP-RP-HPLC (Figure 2)	EE8493 (< QL), EJ0724 (< QL), 00713968-0019-M01 (6%), EJ0701 (17%)	Similar to EG5411, the same late-eluting peaks in IP-RP-HPLC are observed at elevated levels in DP lots with elevated LMS
Nucleoside and nucleotide analysis by LC/MS/MS (Figure 3, Table 3, Table 4)	EE8493 (<QL), EJ0553 (<QL), EJ0724 (<QL), 00713968-019-M01 (6%), EJ0701(17%), EH9978 (35%)	Similar to EG5411, for all tested lots with or without reportable levels of LMS, up to 35%, the expected nucleosides and nucleobases are present, with no detectable modifications greater than 0.01% (reportable limit), besides the intended single 3′-O-methylated 7-methylguanosine and 2′-O-methylated adenosine in the 5′-Cap structure

00713968-019-M01 is a small scale development lot. EH9978 is an early engineering lot. Other DP lots are described in Table 1. QL= quantitation limit (3%)

In addition to the characterization data presented in P.2 on engineering lot EG5411, additional data has been collected by the orthogonal ion-pairing RP-HPLC (IP-RP-HPLC) method presented in P.2 on other representative lots (Figure 2). Similar to EG5411, the lots that have late migrating species by CGE (EJ0701 and 00713968-0019-M01, a small scale development lot with 6% late migrating species) also contain elevated levels of the same late-eluting peaks, as compared to the lots without reported late migrating species (EE8493, EJ0724). As summarized in P.2, the late eluting peaks from IP-RP-HPLC were characterized by UV multi-angle light scattering (MALS) detection, denaturing agarose gel electrophoresis (AGE), and mass spectrometry (MS). The late eluting peaks were characterized as RNA of the expected size that is conformationally folded or reversibly aggregated RNA and is not denatured in the sample preparation of the CGE method.

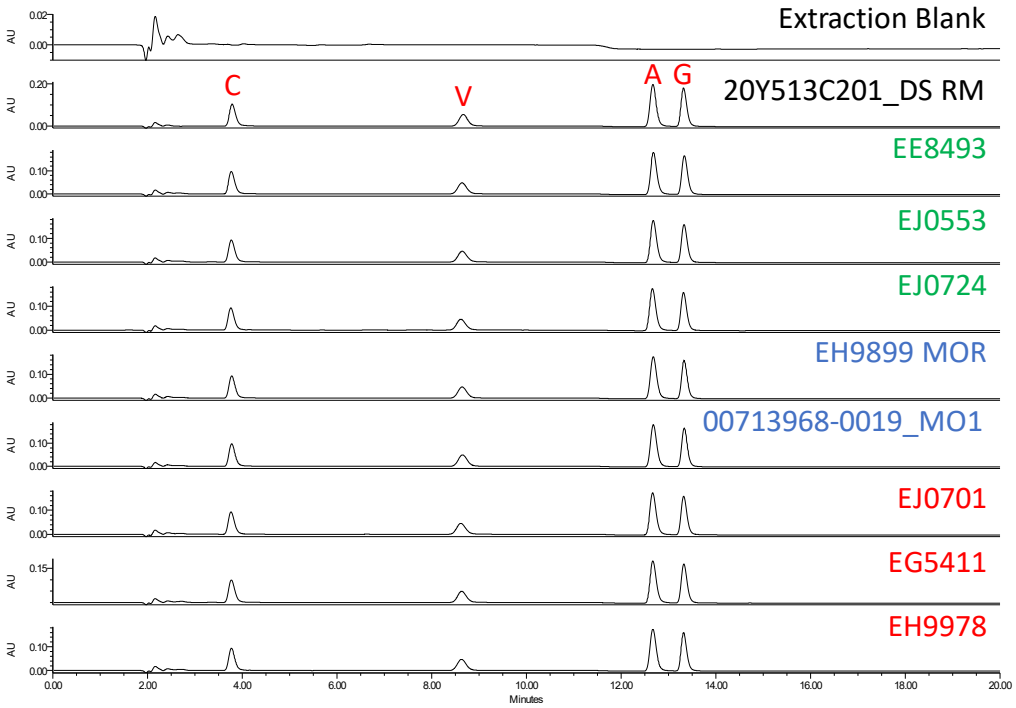
Figure 2. Ion Pairing RP-HPLC of RNA Extracted from Drug Product Lots



DS 20Y513C201-RM is the DS reference material. EH9899 MOR is an in-process sample for the DP lot EH9899. 00713968-019-M01 is a small-scale development lot. Other DP lots are described in Table 1. Based on characterization of the collected IP-RP-HPLC fractions, peak 1 contains fragments, peak 2 contains mostly intact RNA, while peaks 3-5 are correlated with LMS.

In addition to analysis of additional lots by IP-RP-HPLC, nucleoside analysis by LC-UV with online tandem mass spectrometry (MS/MS) was performed on additional lots that have elevated levels of late migrating species, EH9978 and EJ0701, for further confirmation that these lots consist of RNA without detectable modification. Lot EH9978 is an early engineering lot (similar to EG5411) with high levels of LMS (35%); EH9978 does not meet the RNA integrity specification but was used for characterization purposes. Nucleoside analysis involved digestion of DS control and extracted RNA from DP lots with nuclease P1 and venom phosphodiesterase at low and high pH, respectively, and then alkaline phosphatase to remove the phosphate group. The resulting nucleosides were separated by reversed-phase ultrahigh performance liquid chromatography with UV detection at A260 nm using an extended gradient in case of potential modified residues (Figure 3). Each nucleoside displays an elution position and well resolved peak that is consistent between all DP lots and the DS control. The observed accurate monoisotopic masses for each respective peak were consistent with the theoretical masses of the four expected nucleosides from BNT162b2 mRNA (Table 7). The corresponding observed masses of the predominant fragment ions representing the four nucleobases also agreed with the theoretical masses indicating no base modifications (Table 8). These data confirm that like EG5411, a lot with higher levels of late migrating species, that the expected nucleosides and nucleobases are present, with no detectable modifications greater than 0.01% (reportable limit), besides the intended single 3′-O-methylated 7-methylguanosine and 2′-O-methylated adenosine in the 5′-Cap structure (the relative abundances of 2′-O-methylated adenosine in 5′-Cap structure range from 0.24 to 0.42% in the DP lots, similar to DS at 0.37%).

Figure 3. Analysis of Nucleosides in Additional BNT162b2 DP Lots by LC-UV-MS/MS (A260 nm)



BNT162b2 nucleosides: cytidine (C); N1-methylpseudouridine (V); adenosine (A); guanosine (G)

Table 7. Accurate Mass Assignments of Nucleosides for Additional BNT162b2 DP Lots via LC-UV/MS

Nucleoside	Theoretical Mass	DS Reference Material	DP Lots						Engineering Run DP Lots	
		20Y513C201	EE8493	EJ0553	EJ0724	EH9899 MOR	00713968-0019_MO1	EJ0701	EG5411	EH9978
Cytidine (C)	244.0928	244.0925	244.0926	244.0926	244.0926	244.0927	244.0926	244.0925	244.0925	244.0925
N1-methylpseudouridine (V)	259.0925	259.0922	259.0922	259.0921	259.0922	259.0922	259.0923	259.0923	259.0922	259.0923
Adenosine (A)	268.1040	268.1036	268.1036	268.1036	268.1036	268.1037	268.1036	268.1037	268.1037	268.1036
Guanosine (G)	284.0989	284.0988	284.0986	284.0988	284.0987	284.0988	284.0988	284.0988	284.0988	284.0988

Observed masses (monoisotopic) agree with theoretical masses to within 5 ppm, which is consistent with the accuracy and precision of contemporary mass spectrometers

Table 8. Accurate Mass Assignments of Nucleobases for Additional BNT162b2 DP Lots via LC-UV-MS/MS

Nucleobase (derived from respective diagnostic fragment ions)	Theoretical Mass	DS Reference Material	DP Lots						Engineering Run DP Lots	
		20Y513C201	EE8493	EJ0553	EJ0724	EH9899 MOR	00713968-0019_MO1	EJ0701	EG5411	EH9978
Cytosine (C)	112.0511	112.0505	112.0502	112.0503	112.0502	112.0502	112.0503	112.0504	112.0503	112.0504
N1-methylpseudouridine (V)	139.0508	139.0500	139.0499	139.0501	139.0501	139.0500	139.0500	139.0500	139.0500	139.0501
Adenine (A)	136.0623	136.0617	136.0615	136.0615	136.0617	136.0618	136.0615	136.0616	136.0616	136.0616
Guanine (G)	152.0572	152.0564	152.0564	152.0564	152.0564	152.0564	152.0565	152.0565	152.0565	152.0566

Observed masses (monoisotopic) agree with theoretical masses to within 10 ppm, which is consistent with the accuracy and precision of MS/MS in contemporary mass spectrometers

Taken together, these data confirm that the late migrating species observed in additional lots is the same as the EG5411 late migrating species used for detailed characterization, and the additional MS characterization of enzymatically digested RNA confirm the presence of expected nucleosides in additional lots with LMS. The LMS has been characterized as conformationally folded or reversibly aggregated RNA that is not denatured in the CGE method. Lots that are within the RNA integrity specification have comparable in vitro expression as previously shown in P.2, Enhanced Characterization. The CGE method controls both LMS and fragments with a specification limit on the intact RNA species (RNA integrity).

The vaccine drug product is stored at a temperature of -70 °C. Emergency supply DP lots are currently or will be enrolled in formal stability studies (Table 9) . Formal results of these studies will be provided as they become available.

Table 9. Representative Stability Study Design

Study Condition	Storage Temperature	Time Points	Analytical Procedures
Long Term	-90 to -60 °C	0, 1M, 3M, 6M, 9M, 12M, 18M, 24M	• Appearance • Potentiometry • Dynamic Light Scattering (LNP Size Polydispersity) • Fluorescence Assay (RNA Encapsulation, Content) • HPLC-CAD (Lipid Content) • Cell-based Flow Cytometry (In vitro expression) • Capillary Gel Electrophoresis (RNA Integrity) • Subvisible Particles • Container Closure Integrity Test • Endotoxin • Sterility
Accelerated	-60 to -30 °C	0, 1M, 3M, 6M, 9M, 12M, 18M, 24M	
Accelerated	-20 ± 5 °C	0, 1M, 3M, 6M, 9M, 12M, 18M, 24M	
Accelerated	5 ± 3 °C	0, 1M, 3M, 6M	
Thermal Stress	25 ± 2 °C / 60 ± 5 % RH	0, 1W, 2W, 1M	

Current stability data that provide insight on impact of LMS on stability are available from dilution and administration (DAI) simulation studies. Two BNT162b2 DP lots that contain 9-10 % LMS at release (EJ1685 and EJ1688) were evaluated to demonstrate the product has an acceptable RNA integrity at the time of dosing. The DP lots were removed from -70 °C storage, thawed and held in refrigerated storage (2-8 °C) conditions for at least 5 days plus an additional 2 hours at elevated ambient temperatures (30 °C/75% RH). The DP was then diluted to 0.1 mg/mL with normal saline in the vial and held in contact with either the dosing needles and syringes or in the vial for 6 hours at elevated ambient temperatures (30 °C/75% RH). These conditions cover the maximum allowable storage and handling conditions at the point of use. In addition, the diluted DP was held for 24 hours at elevated ambient temperatures (30 °C/75% RH) in a vial to demonstrate the DP meets the specification beyond the allowable conditions.

RNA integrity was acceptable through the allowed administration time period. The RNA integrity data are presented in Table 10.

Additional data on complete DAI study results from two other lots are provided below (Table 11 and Table 12). Similar stability behavior of the RNA integrity level was observed, all results met acceptance criteria and showed acceptable product quality.

In conclusion, the stability profile of the drug product with LMS has been evaluated to confirm acceptable storage and handling through the vaccine point of use, and will continue to be monitored in stability studies. The RNA integrity attribute is a stability indicating assay including for lots that contain LMS, and all lots showed similar stability behavior. The specification for RNA integrity controls both fragment and LMS since both species would lead to lower RNA integrity level. Vials that were thawed for 5 days followed by dilution in saline and a hold at room temperature for up to 6 hours were acceptable; the additional hold of a diluted vial in saline up to 24 hours also met specification including in vitro expression.

Table 10. RNA Integrity Test Results for the Study Performed with Lots# EJ1685[a] and EJ1688[a] (0.1 mg/mL in 0.9% Sodium Chloride)

Test		Acceptance Criteria	T0 (5 days 2-8°C and 2 hours at 30 °C)	30 °C/75% RH				
			Vial	3 Hours Vial	3 Hours Polycarbonate Syringe	6 Hours Vial	6 Hours Polycarbonate Syringe	24 Hours Vial
Lot # EJ1685								
RNA Integrity	Fragment Analyzer (CGE)	T0 Vial ± 20% (≥ 50% Intact RNA)	64.8%	64.7%	64.9%	65.1%	64.0%	63.1%
		Report LMS result	9.7%	9.8%	9.8%	10.1%	10.0%	10.9%
Lot # EJ1688								
RNA Integrity	Fragment Analyzer (CGE)	T0 Vial ± 20% (≥ 50% Intact RNA)	61.4%	59.5%	59.8%	58.4%	58.2%	55.8%
		Report LMS result	11.0%	11.2%	11.9%	12.7%	13.0%	14.4%

a. All vials used in this study were held for ≥5 days at 2-8 °C plus 2 hours at 30 °C prior to dilution
Abbreviations: CGE = capillary gel electrophoresis; LNP = lipid nanoparticle

Table 11. Analytical Test Results for the Study Performed with Lot# EH9899[a] (0.1 mg/mL in 0.9% Sodium Chloride)

Test		Acceptance Criteria	T0 (5 days 2-8°C and 2 hours at 30 °C)	30 °C/75% RH				
			Vial	3 Hours Vial	3 Hours Polycarbonate Syringe	6 Hours Vial	6 Hours Polycarbonate Syringe	24 Hours Vial
Appearance	Visual	White to off-white suspension	Off-white suspension	Off-white suspension	Off-white suspension	Off-white suspension	Off-white suspension	Off-white suspension
	Particulate Matter	Essentially Free of Visible Particles (EFVP)	EFVP	EFVP	EFVP	EFVP	EFVP	EFVP
RNA Content	RiboGreen	T0 Vial ± 20%	0.12 mg/mL	0.12 mg/mL	0.12 mg/mL	0.11 mg/mL	0.12 mg/mL	0.12 mg/mL
RNA Integrity	Fragment Analyzer (CGE)	T0 Vial ± 20% (≥50% Intact RNA)	55.4%	54.2%	54.9%	53.8%	53.3%	50.2%
		Report LMS result	10.0%	11.5%	10.9%	11.4%	11.7%	14.6%
RNA Encapsulation	RiboGreen	≥ 80%	95%	94%	94%	94%	95%	95%
LNP Size	Dynamic Light Scattering	40 to 180 nm	67 nm	67 nm	70 nm	70 nm	69 nm	69 nm
LNP Polydispersity	Dynamic Light Scattering	≤ 0.3	0.16	0.16	0.19	0.19	0.18	0.17
In-Vitro Expression	Cell-based Flow Cytometry	≥ 30% positive cells	86%	76%	69%	79%	77%	69%

a. All vials used in this study were held for ≥5 days at 2-8 °C plus 2 hours at 30 °C prior to dilution
Abbreviations: CGE = capillary gel electrophoresis; LNP = lipid nanoparticle

Table 12. Analytical Test Results for the Study Performed with Lot# EJ0553[a] (0.1 mg/mL in 0.9% Sodium Chloride)

Test		Acceptance Criteria	T0 (5 days 2-8°C and 2 hours at 30 °C)	30 °C/75% RH				
			Vial	3 Hours	3 Hours	6 Hours	6 Hours	24 Hours
				Vial	Polycarbonate Syringe	Vial	Polycarbonate Syringe	Vial
Appearance	Visual	White to off-white suspension	Off-white suspension	Off-white suspension	Off-white suspension	Off-white suspension	Off-white suspension	Off-white suspension
	Particulate Matter	Essentially Free of Visible Particles (EFVP)	EFVP	EFVP	EFVP	EFVP	EFVP	EFVP
RNA Content	RiboGreen	T0 Vial ± 20%	0.10 mg/mL	0.11 mg/mL	0.10 mg/mL	0.10 mg/mL	0.11 mg/mL	0.11 mg/mL
RNA Integrity	Fragment Analyzer (CGE)	T0 Vial ± 20% (≥ 50% Intact RNA)	67.2%	67%	66.3%	66.1%	65.6%	64.2%
		Report LMS result	2.5%	2.3%	2.7%	2.6%	2.4%	2.8%
RNA Encapsulation	RiboGreen	≥ 80%	93%	92%	92%	92%	94%	93%
LNP Size	Dynamic Light Scattering	40 to 180 nm	69 nm	69 nm	74 nm	70 nm	68 nm	76 nm
LNP Polydispersity	Dynamic Light Scattering	≤ 0.3	0.18	0.17	0.18	0.17	0.15	0.19
In-Vitro Expression	Cell-based Flow Cytometry	≥ 30% positive cells	79%	63%	75%	74%	71%	82%

a. All vials used in this study were held for ≥5 days at 2-8 °C plus 2 hours at 30 °C prior to dilution
Abbreviations: CGE = capillary gel electrophoresis; LNP = lipid nanoparticle

REGARDING THE INTRINSIC PARTICLES OBSERVED DURING VISUAL INSPECTION, PLEASE PROVIDE THE FOLLOWING INFORMATION:

QUERY 8

Please clarify the percentage of DP lots that contain the intrinsic visible particles and whether particles occurred only in the most recent DP lots. Please comment on the possible contributing factors that may lead to the formation of these particles.

RESPONSE 8

Table 13 (see Query 9) presents information for drug product lots and includes the inspection method and the percentage of vials that were rejected due to particles during inspection. As can be seen, in 29 out of 33 lots filled and inspected at the Pfizer Puurs and Pfizer Kalamazoo sites including early lots produced, a small number of vials were detected during visual inspection and rejected from the lot due to particles (min 0.01% - max 3.68%). In Pfizer's experience with suspension vaccine products, these rejection rates are not uncommon. The particles specifically from EJ0553, EG5411 (bulk DP lot EG5477, Pfizer Puurs) and EH9899 (bulk DP lot EH9783 Pfizer Kalamazoo) were characterized and identified as product related and most likely comprised of lipids and cholesterol components, as assessed by FTIR spectra. When a vial with particles was diluted with 1.8 mL of sterile 0.9% sodium chloride, Inj., mixed and the resulting solution drawn into a syringe, the particles appeared to disperse.

No specific factors have been identified as definitively correlating with nor directly contributing to the formation of these intrinsic particles. Particles have been observed to a varying degree across many lots spanning multiple manufacturing sites, including four sites of LNP production and two fill finish sites, as listed in Table 13 and across different lipid sources (vendors and batches) as listed in Table 14. Particles have been observed in the sterile holding vessel post-sterile filtration and before aseptic filling. Particles are light in density and have a tendency to float. Because sterile bulk drug product flows from the bottom of the vessel to the filling line, floating particles tend to be observed more frequently in vials filled towards the end of the filling process.

Literature References

None

SUPPORTING DOCUMENTATION

None

QUERY 9

For all lots that contain the visible intrinsic particles, please submit data on the percentage of vials that contain the intrinsic particles (for example, through data obtained from the 100% automated/manual inspection).

RESPONSE 9

Previously submitted Section P.2 Pharmaceutical Development, Table P.2-17 Drug Product Lot Genealogy and Usage is amended below as Table 13 with the inspection method (100% manual or 100% automated) and the percentage of vials that were rejected due to particles during inspection. Information is presented for lots where fill finish was conducted at either Pfizer Puurs or Pfizer Kalamazoo which are the fill finish sites intended for manufacture of emergency supply and includes lots that are not intended for emergency supply (but are manufactured at the same sites using the essentially the same process). Vials that are rejected due to particles during 100% visual inspection are not reintroduced into the batch and are discarded. The acceptance quality limit (AQL) sampling procedure assesses the robustness of the inspection method, as a statistically determined number of vials that passed the inspection method are reinspected manually. The appearance method and label reflect that after dilution, in the rare case particles are observed prior to administration, the product should not be administered.

Table 13. Lot Genealogy and Vials Rejected During Visual Inspection for Particles

DP Lot Number (DP Name)	Fill Volume (mL)	Drug Product (DP)		Date of Manufacture	Manufacturing Scale (vials)	Drug Substance Site of Manufacture	Inspection method	% vials rejected during inspection due to particles
		LNP Site (Process)	Fill/Finish Site					
CTM10.4 BCV40720-P/ ED3938	0.2	Polymun Scientific (Classical)	Pfizer Puurs (S2F2)	16 Jul 2020	19,010	BioNTech	100% Manual Visual Inspection	0.06%
CTM11 BCV40820-P/ EE3813	0.2	Polymun Scientific (Classical)	Pfizer Puurs (S2F2)	29 Jul 2020	30,193	BioNTech	100% Manual Visual Inspection	0.02%
CTM12 BCV4/L05 EE8492	0.45	Polymun Scientific (Upscale)	Pfizer Puurs (WSL5)	05 Aug 2020	67,665	Pfizer Andover	100% automated inspection at IL7	0.41%
CTM13 BCV4/L06 EE8493	0.45	Polymun Scientific (Upscale)	Pfizer Puurs (WSL5)	05 Aug 2020	68,445	Pfizer Andover	100% automated inspection at IL7	0.06%
CTM14 BCV4/L07 EJ0553	0.45	Polymun Scientific (Upscale)	Pfizer Puurs (WSL5)	25 Sep 2020	164,580	Pfizer Andover	100% automated inspection at IL7	0.34%
BCV4/L08 EJ1685	0.45	Polymun Scientific (Upscale)	Pfizer Puurs (WSL5)	05 Oct 2020	159,315	BioNTech; Rentschler	100% automated inspection at IL7	0.16%
BCV4/L09 EJ1686 (BNT162b2)	0.45	Polymun Scientific (Upscale)	Pfizer Puurs (WSL5)	07 Oct 2020	147,615	BioNTech; Rentschler	100% automated inspection at IL7	0.04%
BCV4/L10 EK1768 (BNT162b2)	0.45	Polymun Scientific (Upscale)	Pfizer Puurs (WSL5)	16 Oct 2020	141,960	Pfizer Andover	100% automated inspection at IL7	0.30%
EG5411	0.45	Pfizer Puurs	Pfizer Puurs (FC2)	3 Sep 2020	201,258	Pfizer Andover	100% automated inspection at Innoscan	0.29%
EJ0701	0.45	Pfizer Kalamazoo	Pfizer Kalamazoo (Line 18)	26 Sep 2020	200,265	Pfizer Andover	100% Manual Visual Inspection	0%
EH9978	0.45	Pfizer Puurs	Pfizer Puurs (FC2)	23 Sept 2020	304,869	Pfizer Andover	100% automated inspection at Innoscan	1.30%

Table 13. Lot Genealogy and Vials Rejected During Visual Inspection for Particles

DP Lot Number (DP Name)	Fill Volume (mL)	Drug Product (DP)		Date of Manufacture	Manufacturing Scale (vials)	Drug Substance Site of Manufacture	Inspection method	% vials rejected during inspection due to particles
		LNP Site (Process)	Fill/Finish Site					
EJ0724	0.45	Dermapharm	Pfizer Puurs (WSL5)	29 Sep 2020	39,195	BioNTech; Rentschler	100% automated inspection at IL7	0.01%
EH9899	0.45	Pfizer Kalamazoo	Pfizer Kalamazoo (Line 8)	7 Oct 2020	179,400	Pfizer Andover	100% automated inspection	0.01%
EJ1688	0.45	Dermapharm	Pfizer Puurs (WSL5)	12 Oct 2020	150.345	BioNTech; Rentschler	100% automated inspection at IL7	0.06%
EK4176	0.45	Polymun	Pfizer Puurs (WSL5)	16 Oct 2020	131.625	BioNTech; Rentschler	100% automated inspection at IL7	0.03%
EK4175	0.45	Dermapharm	Pfizer Puurs (WSL5)	12 Oct 2020	145.275	BioNTech; Rentschler	100% automated inspection at IL7	0.09%
EJ1691	0.45	Dermapharm	Pfizer Puurs (WSL5)	16 Oct 2020	133.575	BioNTech Rentschler	100% automated inspection at IL7	1.17%
EK2808	0.45	Puurs	Pfizer Puurs (VC2)	19 Oct 2020	48,945	BioNTech Rentschler	100% automated inspection at Innoscan	0.57%
EK5730	0.45	Pfizer Kalamazoo	Pfizer Kalamazoo (Line 8)	22 Oct 2020	191,295	Pfizer Andover	100% automated inspection	0.09%
EL0140	0.45	Dermapharm	Pfizer Puurs (WSL5)	29 Oct 2020	155.610	BioNTech; Rentschler	100% automated inspection at IL7	0.52%
EL0142	0.45	Dermapharm	Pfizer Puurs (WSL5)	29 Oct 2020	138.060	BioNTech; Rentschler	100% automated inspection at IL7	0.81%
EL0141	0.45	Polymun	Pfizer Puurs (WSL5)	29 Oct 2020	156.195	BioNTech; Rentschler	100% automated inspection at IL7	0.15%
EL0725	0.45	Puurs	Pfizer Puurs (FC2)	30 Oct 2020	272,073	BioNTech; Rentschler	100% automated inspection at Innoscan	1.20%
EK9231	0.45	Pfizer Kalamazoo	Pfizer Kalamazoo (Line 18)	4 Nov 2020	230,685	Pfizer Andover	100% automated inspection	0.02%
EK4237	0.45	Dermapharm	Pfizer Puurs (WSL5)	5 Nov 2020	140.985	BioNTech; Rentschler	100% automated inspection at IL7	3.68%
EL0739	0.45	Puurs	Pfizer Puurs (FC2)	3 Nov 2020	294,239	BioNTech; Rentschler	100% automated inspection at Innoscan	1.34%

Table 13. Lot Genealogy and Vials Rejected During Visual Inspection for Particles

DP Lot Number (DP Name)	Fill Volume (mL)	Drug Product (DP)		Date of Manufacture	Manufacturing Scale (vials)	Drug Substance Site of Manufacture	Inspection method	% vials rejected during inspection due to particles
		LNP Site (Process)	Fill/Finish Site					
EL1484	0.45	Puurs	Pfizer Puurs (FC2)	4 Nov 2020	277,608	BioNTech; Rentschler	100% automated inspection at Innoscan	0.58%
EL1283	0.45	Pfizer Kalamazoo	Pfizer Kalamazoo (Line 18)	11 Nov 2020	245,895	Pfizer Andover	100% automated inspection	0%
EL1284	0.45	Pfizer Kalamazoo	Pfizer Kalamazoo (Line 18)	17 Nov 2020	214,305	Pfizer Andover	100% automated inspection	0%
EL3246	0.45	Pfizer Kalamazoo	Pfizer Kalamazoo (Line 8)	19 Nov 2020	204,360	Pfizer Andover	100% automated inspection	0%
EJ6795	0.45	Puurs	Pfizer Puurs (FC2)	12 Nov 2020	282.645	Pfizer Andover	100% automated inspection at Innoscan	0.89%
EJ6796	0.45	Puurs	Pfizer Puurs (FC2)	13 Nov 2020	293,828	Pfizer Andover	100% automated inspection at Innoscan	0.58%
EJ6797	0.45	Puurs	Pfizer Puurs (FC2)	17 Nov 2020	293.526	Pfizer Andover	100% automated inspection at Innoscan	0.48%

Literature References

None

SUPPORTING DOCUMENTATION

None

Previously submitted supporting documentation

Section P.2 Pharmaceutical Development (modRNA), SN0137

QUERY 10

Please identify the source of the lipids used for the impacted DP lots.

RESPONSE 10

Previously submitted Section P.2 Pharmaceutical Development, Table P.2-18. Lipids Used in BNT162b2 Drug Product Manufacture is amended below as Table 14 with the inspection method and the percentage of vials that were rejected due to particles during inspection (same data as in Table 13). It now lists for each DP lot, the lipid lots used during manufacturing, the related inspection method and percentage of vials rejected due to particles. No correlation is observed between the frequency of vials with particles and the lipid lots used.

Table 14. Lipids Used in BNT162b2 Drug Product Manufacture[a]

Drug Product Lot	ALC-0315		ALC-0159		DSPC		Cholesterol		Inspection method	% vials rejected during inspection due to particles
	Manufacturer	Lot Number	Manufacturer	Lot Number	Manufacturer	Lot Number	Manufacturer	Lot Number		
BCV40720-P /ED3938	Avanti	GALC0315-10 GALC0315-11	Avanti	GALC0159-11	Lipoid	556500-2190388-01	Wilshire[b]	P60349	100% Manual Visual Inspection	0.06%
BCV40820-P /EE3813	Avanti	GALC0315-12	Avanti	GALC0159-11	Lipoid	556500-2190395-01	Wilshire	P60349	100% Manual Visual Inspection	0.02%
BCV4/L05 /EE8492	Avanti	GALC0315-12	Avanti	GALC0159-12	Lipoid	556500-2190395-01	Wilshire	P60349	100% automated inspection at IL7	0.41%
BCV4/L06 /EE8493	Avanti	GALC0315-13	Avanti	GALC0159-12	Lipoid	556500-2190395-01	Wilshire	P90390	100% automated inspection at IL7	0.06%
BCV4/L07 /EJ0553	Avanti	GALC0315-12/ GALC0315-13	Avanti	GALC0159-12	Lipoid	556500-2190395-01	Wilshire	P90390	100% automated inspection at IL7	0.34%
BCV4/L08 /EJ1685	Croda	DTP/465/3	Avanti	GALC0159-12	Lipoid	556500-2180372-01 556500-2200421-01	Wilshire	P90390	100% automated inspection at IL7	0.16%
BCV4/L09 /EJ1686	Croda	DTP/465/3	Avanti	GALC0159-12/ALC0159-104	Lipoid	556500-2200421-01	Wilshire	P90390	100% automated inspection at IL7	0.04%

Table 14. Lipids Used in BNT162b2 Drug Product Manufacture[a]

Drug	ALC-0315		ALC-0159		DSPC		Cholesterol		Inspection	% vials
BCV4/L10 /EK1768	Croda	1755889	Avanti	ALC0159-104	Lipoid	556500-2200421-01	Wilshire	P90390	100% automated inspection at IL7	0.30%
EG5411	Croda	DTP/465/1	Avanti	GALC0159-12	Avanti	DSPCIIS-111	Avanti	SCHOLB-105	100% automated inspection at Innoscan	0.29%
EJ0701	Croda	DTP/465/1	Avanti	ALC0159-105	Avanti	DSPCIIS-112	Avanti	SCHOLB-105	100% Manual Visual Inspection	0%
EH9978	Croda	DTP/465/1	Avanti	GALC0159-12	Avanti	DSPCIIS-111	Avanti	SCHOLB-105	100% automated inspection at Innoscan	1.30%
EJ0724	Avanti	GALC0315-14	Avanti	ALC0159-105	Avanti	DSPCIIS-111	Avanti	SCHOLB-105	100% automated inspection at IL7	0.01%
EH9899	Croda	DTP/465/3	Avanti	ALC0159-105	Avanti	DSPCIIS-112	Avanti	SCHOLB-105	100% automated inspection	0.01%
EJ1688	Croda	1755889	Avanti	ALC0159-105	Avanti	DSPCIIS-111	Avanti	SCHOLB-105	100% automated inspection at IL7	0.06%
EK4176	Croda	1760275	Avanti	ALC0159-104	Lipoid	556500-2200421-01	Wilshire	P90390	100% automated inspection at IL7	0.03%
EK4175	Croda	1760275	Avanti	ALC0159-106	Avanti	DSPCIIS-111	Avanti	SCHOLB-105	100% automated inspection at IL7	0.09%
EJ1691	Croda	1760275	Avanti	ALC0159-106	Avanti	DSPCIIS-111	Avanti	SCHOLB-105	100% automated inspection at IL7	1.17%

Table 14. Lipids Used in BNT162b2 Drug Product Manufacture[a]

Drug	ALC-0315		ALC-0159		DSPC		Cholesterol		Inspection	% vials
EK2808	Croda	1760275	Avanti	ALC0159-104	Avanti	DSPCIIS-111	Avanti	SCHOLB-105	100% automated inspection at Innoscan	0.57%
EK5730	Croda	DTP/465/3	Avanti	ALC0159-105	Avanti	DSPCIIS-112	Avanti	SCHOLB-105	100% automated inspection	0.09%
EL0140	Croda	1755889	Avanti	ALC0159-106	Avanti	DSPCIIS-111	Avanti	SCHOLS-129	100% automated inspection at IL7	0.52%
EL0142	Croda	1755889	Avanti	ALC0159-106	Avanti	DSPCIIS-111	Avanti	SCHOLS-129	100% automated inspection at IL7	0.81%
EL0141	Croda	1755889	Avanti	ALC0159-104	Lipoid	556500-2200421-01	Wilshire	P90390	100% automated inspection at IL7	0.15%
EL0725	Croda	DTP/465/3	Avanti	ALC0159-106	Avanti	DSPCIIS-112	Avanti	SCHOLS-129	100% automated inspection at Innoscan	1.20%
EK9231	Croda	DTP/465/3	Avanti	ALC0159-105	Avanti	DSPCIIS-112	Avanti	SCHOLB-105	100% automated inspection	0.02%
EK4237	Croda	1755889, 1760275	Avanti	ALC0159-107	Avanti	DSPCIIS-111	Avanti	SCHOLS-129	100% automated inspection at IL7	3.68%
EL0739	Croda	DTP/465/3	Avanti	ALC0159-106	Avanti	DSPCIIS-112	Avanti	SCHOLB-105	100% automated inspection at Innoscan	1.34%
EL1484	Croda	DTP/465/3	Avanti	ALC0159-106	Avanti	DSPCIIS-112	Avanti	SCHOLB-105	100% automated inspection at Innoscan	0.58%

Table 14. Lipids Used in BNT162b2 Drug Product Manufacture[a]

Drug	ALC-0315		ALC-0159		DSPC		Cholesterol		Inspection	% vials
EL1283	Croda	DTP/465/3	Avanti	ALC0159-105	Avanti	DSPCIIS-112	Avanti	SCHOLB-105	100% automated inspection	0%
EL1284	Croda	1760275	Avanti	ALC0159-105	Avanti	DSPCIIS-112	Avanti	SCHOLB-105	100% automated inspection	0%
EL3246	Croda	1760275	Avanti	ALC0159-105	Avanti	DSPCIIS-112	Avanti	SCHOLS-129	100% automated inspection	0%
EJ6795	Croda	1760275	Avanti	ALC0159-104	Avanti	DSPCIIS-112	Avanti	SCHOLB-105	100% automated inspection at Innoscan	0.89%
EJ6796	Croda	1760275, DTP/465/3	Avanti	ALC0159-104	Avanti	DSPCIIS-112	Avanti	SCHOLB-105	100% automated inspection at Innoscan	0.58%
EJ6797	Croda	0001781853	Avanti	ALC0159-106	Avanti	DSPCIIS-111	Avanti	SCHOLS-129	100% automated inspection at Innoscan	0.48%

a. Only lipids used for BNT162b2 manufacture are listed as BNT162b1 is no longer under development.
b. Wilshire is now Evonik.

Literature References

None

SUPPORTING DOCUMENTATION

New or Replaced Supporting Documentation

None

Previously submitted supporting documentation

Section P.2 Pharmaceutical Development (modRNA), SN0137

QUERY 11

For DP lots with visible particles, please provide batch analysis data and stability data to support that there is no impact on DP quality.

RESPONSE 11

As described in the responses to Query 9 and Query 10, visible particles have been observed during 100% inspection in a small number of vials for nearly all DP lots. Therefore, the drug product batch analysis data and stability data previously submitted to the IND are inclusive of lots with visible particles. Future emergency supply DP lots will also be enrolled in formal stability studies and results of all studies will be provided as they become available.

Literature References

None

SUPPORTING DOCUMENTATION

None

REGARDING THE DS MANUFACTURING PROCESS:

QUERY 12

At BNT, there will be a hold/transportation step at 2-8°C (from Mainz to Rentschler). However, the duration of this step ("≥ 96") is not accurate. Please specify the duration of this step.

RESPONSE 12

We acknowledge this inconsistency in the previous submission. The hold/transportation time of the Proteinase K Pool is ≤ 96 h. The corresponding Table S.2.2.-4 in Section 3.2.S.2.2 Description of Manufacturing Process and Process Controls (modRNA) [BNT Mainz and Rentschler] has been corrected.

Literature References

None

SUPPORTING DOCUMENTATION

New or Replaced Supporting Documentation

3.2.S.2.2 Description of Manufacturing Process and Process Controls (modRNA) [BNT Mainz and Rentschler], Replaced

Previously submitted supporting documentation

None

QUERY 13

Regarding the DS manufacture at Pfizer, Andover, please specify the conditions and length of hold time for the filtered DS before being dispensed into EVA containers, if applicable.

RESPONSE 13

The UFDF pool undergoes a bulk final 0.45/0.2 µm filtration into a flexible container. The filtered DS is then mixed, sampled and dispensed into EVA containers. The whole process from bulk filtration to end of dispense into EVA containers takes less than 24 hours as described in Section 3.2.S.2.5 Process Validation and/or Evaluation - Hold Times [Andover]. This is considered continuous processing and not evaluated as hold time.

Literature References

None

SUPPORTING DOCUMENTATION

New or Replaced Supporting Documentation

None

Previously submitted supporting documentation

Section S.2.5 Process Validation and/or Evaluation - Hold Times (modRNA) [Andover], SN0137

QUERY 14

Please describe the target concentrations (both in terms of U/mL and mg/mL) for the active proteins (DNase I, proteinase K, pyrophosphatase, RNase inhibitor, and T7 polymerase) used in the In Vitro Transcription, DNase I Digestion, and Proteinase K Digestion steps.

RESPONSE 14

In Table 15 below, the vendor specifications are provided, as well as the target concentration in the reaction for the active proteins used in the In Vitro Transcription, DNase I Digestion and Proteinase K digestion steps. The vendor formulates the active proteins in units (U) per volume. The concentration in mg/mL is provided by the vendor as report results in CoA for a subset of these enzymes and has not been used to set target concentration for the active proteins. Volumes for the active proteins that are defined in the filing were determined using the target concentration and the target value in the vendor specification.

Below, the vendor specifications are provided, as well as the target concentration in the reaction for the active proteins used in the In Vitro Transcription, DNase I Digestion and Proteinase K digestion steps. The vendor formulates the active proteins in units (U) per volume. The concentration in mg/mL is provided by the vendor as report results in CoA for a subset of these enzymes and has not been used to set target concentration for the active proteins. Target concentrations for the active proteins are defined based on the vendor specifications for activity.

Table 15. Active Proteins Vendor Specification and Target Concentration During In Vitro Transcription, DNase I Digestion and Proteinase K Steps

Raw material	Vendor Specification (U/µL solution)	Target Concentration (U/ L starting IVT volume)
RNase inhibitor	40 ± 6	50,000
Pyrophosphatase	0.10 ± 0.02	100
T7 polymerase	200 ± 21	16,000,000
DNase I	50 ± 9	400,000
Proteinase K	≥ 0.600	676

Literature References

None

SUPPORTING DOCUMENTATION

None

QUERY 15

Please provide a description of the process controls implemented during the sanitization, equilibration, and reuse procedure for the ultrafiltration/diafiltration (UFDF) membrane.

RESPONSE 15

The Pfizer, Andover and Rentschler, Laupheim membrane sanitization, equilibration, and reuse procedures are provided below. Process controls described in this section are included in a membrane lifetime protocol that will be provided for the BLA submission.

Pfizer, Andover

Sanitization
For sanitization, an initial WFI flush is performed followed by a 0.5 M NaOH solution flush and recirculation for 60 min through the membrane. Following this sanitization, a subsequent WFI flush is performed prior to determining the normalized water permeability (NWP). The membranes are then stored in 0.1 N NaOH.

Equilibration
Prior to UFDF execution, a WFI flush is performed to remove the 0.1 N NaOH storage solution. Following the WFI flush, the membranes are equilibrated with equilibration buffer. As shown in Table 16, bioburden and endotoxin samples are pulled from the retentate line after equilibration is completed to demonstrate effective microbial control during membrane storage. Furthermore, pH and conductivity are tested for retentate and permeate after equilibration.

Reuse
Membranes intended for re-use are flushed with DS formulation buffer after use and sanitized as described above.

Table 16 below tabulates the process controls implemented for membrane reuse at commercial scale.

Table 16. Routine Process Controls Implemented During the Sanitization, Equilibration, and Reuse procedure (Pfizer, Andover)

Membrane Performance Test
Normalized membrane permeability post commissioning or cleaning (% recovery)
Equilibration retentate: bioburden (CFU/10mL)[a]
Equilibration retentate: endotoxin (EU/mL)[a]
Post diafiltration 2 permeate conductivity (mS/cm)
Post diafiltration 2 permeate pH
UFDF retentate pre-recovery product pool: bioburden (CFU/10mL)
UFDF retentate pre-recovery product pool: endotoxin (EU/mL)
Drug substance residual DNA template (ng DNA/mg RNA)
Step yield (%)

Abbreviations: CFU = colony forming units; EU = endotoxin units; UFDF = ultrafiltration/diafiltration; WFI = water for injection
a. Testing is performed as part of membrane lifetime validation and the testing frequency may be changed after validation is complete

Rentschler, Laupheim

Sanitization
The TFF system and membrane are sanitized pre- and post-use. If the membrane was used before, samples for bioburden and endotoxins are pulled between end of storage and pre-use sanitization.

For sanitization, an initial WFI flush is performed followed by a 1.0 M NaOH solution flush and recirculation for 60 – 65 min through the membrane. Subsequently, the normalized membrane permeability (NMP) is measured. For NMP 0.1 M NaOH is used. The membranes are then stored in 0.1 N NaOH.

Equilibration
Prior to UFDF batch execution, the membranes are equilibrated with equilibration buffer. As shown in Table 17, bioburden and endotoxin samples are pulled from the retentate and permeate line after equilibration is completed to demonstrate effective microbial control during membrane storage. Furthermore, pH and conductivity are tested for retentate and permeate after equilibration.

Reuse
Membranes intended for re-use are flushed with DS formulation buffer after use and sanitized as described above.

Table 17 below tabulates the process controls implemented for membrane reuse at commercial scale.

Table 17. Routine Process Controls Implemented During the Sanitization, Equilibration, and Reuse Procedure

Step	Process controls
Sanitization	Post storage retentate and permeate: bioburden (CFU/10mL)
	Post storage retentate and permeate: endotoxins (EU/mL)
	Normalized membrane permeability post commissioning (%)
	Pre-use: membrane integrity test
Equilibration	Post equilibration retentate and permeate: bioburden (CFU/10mL)
	Post equilibration retentate and permeate: endotoxins (EU/mL)
	Post equilibration retentate and permeate: pH
	Post equilibration retentate and permeate: conductivity (mS/cm)
Reuse	Normalized membrane permeability post use (%)
	Pre storage: bioburden (CFU/10mL)
	Pre storage: endotoxins (EU/mL)
	Pre storage retentate and permeate: conductivity (mS/cm)

Abbreviations: CFU = colony forming units; EU = endotoxin units;

Literature References

None

SUPPORTING DOCUMENTATION

None

QUERY 16

Please note that all drug substance process steps must be performed under cGMP conditions. Data to support complete process validation under cGMP conditions must be available at the time of BLA submission.

RESPONSE 16

The sponsor confirms that all drug substance process steps are performed under cGMP conditions. Complete data process validation details will be provided in the BLA.

Literature References

None

SUPPORTING DOCUMENTATION

None

REGARDING THE DP MANUFACTURING PROCESS:

QUERY 17

Please provide a description of the process controls implemented during the sanitization, equilibration, and reuse procedure for the tangential flow filtration (TFF) membrane.

RESPONSE 17

Sanitization/Equilibration

Polymun, Austria and DermaPharm, Germany

Sanitization/Equilibration

The TFF membranes used in the drug product (DP) manufacturing process are currently used once and then discarded. For Polymun, these filters are not supplied gamma irradiated/pre-sterilized. However, for DermaPharm, these filters arrive gamma irradiated/pre-sterilized. In both cases, the filters are sanitized by a flush and recirculation through the TFF membranes with 0.1 N sodium hydroxide (NaOH). The filters are then equilibrated for at least 24 hours with the same NaOH buffer. If the TFF membranes are not used after 24 hours of the initial NaOH sanitization step, the TFF membranes can stored in 0.1 N NaOH at 2-8 °C.

After this sanitization step, the filters are rinsed with WFI until conductivity of the permeate stream reaches the conductivity of the WFI. For equilibration and pre-conditioning prior to use in DP processing, a citrate buffer flush and recirculation are then performed until a permeate pH of 3.9-4.1 is achieved.

Reuse

A regeneration/reuse process for the TFF membranes in the DP manufacturing process is not currently planned.

Pfizer Puurs, Belgium and Pfizer Kalamazoo, MI, US

Sanitization/Equilibration

The TFF membranes used in the DP manufacturing process are currently used once and then discarded. These filters arrive gamma irradiated/pre-sterilized. As such, a sanitization is not required prior to use. However, a flush/recirculation through the TFF membrane is performed with WFI or citrate buffer, after which a pre-use integrity test is performed with WFI or citrate buffer. If citrate buffer was not used in the prior step, an additional citrate buffer flush and recirculation is performed for equilibration. The TFF membranes are then used for DP processing.

Reuse

A regeneration/reuse process for the TFF membranes is targeted for implementation in GMP manufacturing of drug product in December 2020. The implementation of a regeneration/reuse procedure will include additional controls including TFF membrane cleaning with ≥20% isopropyl alcohol (IPA), WFI and NaOH and a subsequent pre-use sanitization with NaOH as well as additional sampling and testing through a concurrent validation protocol to ensure appropriate membrane performance and microbial and residual carryover control. Performance tests for the concurrent validation protocol for membrane reuse will include but not be limited to those shown in Table 18.

Table 18. Performance Tests Implemented for Concurrent Validation for Membrane Regeneration and Reuse

Membrane Performance Test
Normalized water permeability post commissioning or cleaning (% recovery)
Membrane integrity test
Post-CIP: bioburden
Post-CIP: endotoxin
Post TFF: bioburden (pre-bioburden reduction filtration)[a]
Post TFF: endotoxin (pre-bioburden reduction filtration)[a]
Yield (%)

a. Routine in-process test
Abbreviations: CFU = colony forming units; CIP = clean in place; EU = endotoxin units; TFF = tangential flow filtration

Literature References

None

SUPPORTING DOCUMENTATION

None

REGARDING THE VALIDATION OF ANALYTICAL PROCEDURES:

QUERY 18

In 2.3.1 Introduction p. 4 (Amendment 138; submitted on November 16, 2020), you stated that "the same method and validation information apply to each testing location". However, the summaries of qualification/validation study results provided thus far (3.2.S.4.3 and 3.2.P.5.3) do not appear to contain site-specific information. Please explicitly specify that the same critical reagents are used for each testing site. If so, please provide data to demonstrate comparable assay performance between testing sites. If different SOPs/critical reagents are used, please provide additional assay validation data.

RESPONSE 18

Additional method validation data are being provided for the BioNTech/Rentschler sites. The test method standard operating procedure numbers for drug substance and drug product testing are listed in Table 19 and Table 20 below. The Pfizer Andover and Chesterfield laboratories perform all drug product testing and drug substance testing for DS manufactured at Pfizer, Andover. The Pfizer laboratories use the same standard operating procedures. Both laboratories were included in the co-validation of the test methods, and the intermediate precision determined through the reproducibility studies were suitable to demonstrate consistent performance. The compendial method verifications were performed at the Chesterfield or Andover site. The Pfizer validation data were provided in Section S.4.3 Validation of Analytical Procedures (modRNA) of SN0137 (submitted 16 November 2020). The BioNTech and Rentschler laboratories perform release and stability testing for drug substance manufactured at BioNTech/Rentschler sites. The site and method of validation are provided in Table 19. The BioNTech/Rentschler validation data are presented in Table 21 to Table 26 (excluding the BioNTech immunoblot validation for dsRNA, which was provided in a previous amendment). For methods which use critical reagents, the same lot or an alternate qualified lot is used across testing sites. The intermediate precision determined in the method validation studies are used to demonstrate consistent assay performance between sites. As indicated in Table 19, all drug substance 5'-Cap and Poly(A) tail testing are performed by Pfizer; all dsRNA testing is performed by BioNTech.

Table 19. Drug Substance Testing

Quality Attribute	Analytical Procedure	Pfizer			BioNTech/Rentschler		
		Test Method Procedure Number	Verification or Validation Report #	Site Participating in Validation	Test Method Procedure Number	Verification or Validation Report #	Site Participating in Validation
Clarity, Coloration	Appearance	TM100010539	VAL100131145	Verification of compendia	RL-SOP-00984/ RL-SOP-00994	RL-Statement-00084	Rentschler
pH	Potentiometry	TM100010538	VAL100131145	Verification of compendia	RL-SOP-00995	RL-Statement-00084	Rentschler
Content (RNA Concentration)*	Platform UV Spectroscopy	TM100010308	VAL100188111 VAL100121467	Chesterfield, MO	RL-SOP-02645	RL-Report -09484	Rentschler
Identity of Encoded RNA Sequence	RT-PCR	TM100010407	VAL100121706	Co-validation performed at Andover, MA and Chesterfield, MO	TM-072-038	MVR-20-0017	BioNTech, Mainz
RNA Integrity	Capillary Gel Electrophoresis	TM100010392	VAL100122020	Co-validation performed at Andover, MA and Chesterfield, MO	TM-072-039	MVR-20-0018	BioNTech, Mainz
5' - Cap	RP-HPLC	TM100010578	VAL100123137	Co-validation performed at Andover, MA and Chesterfield, MO	NA	NA	NA
Poly(A) Tail	ddPCR	TM100010379	VAL100123048	Co-validation performed at Andover, MA and Chesterfield, MO	NA	NA	NA
Residual Template DNA	qPCR	TM100010388	VAL100121343	Co-validation performed at Andover, MA and Chesterfield, MO	TM-072-028	MVR-20-0007	BioNTech, Mainz
dsRNA	Immunoblot	NA	NA	NA	PAN-0720-K	VAL-2083-VB	BioNTech, IMFS
Bacterial Endotoxin*	Endotoxin (LAL)	TM100010753	VAL100121189	Andover, MA	RL-SOP-00860	RL-Report-09494	Rentschler
Bioburden*	Bioburden	TM100010714	VAL100121185	Andover, MA	RL-SOP-00858	RL-Report-09493	Rentschler

*Performed at Andover, MA and Rentschler as an IPC during DS manufacturing

Table 20. Drug Product Testing

Quality Attribute	Analytical Procedure	Test Method Procedure Number	Verification or Validation Report #	Site Participating in Validation
Appearance (Visual, Particles)	Appearance	TM100010539	VAL100131145	Verification of compendia
Subvisible Particles	Subvisible particulate matter	TM100010541	VAL100131145 VAL100126212	Verification of compendia
pH	Potentiometry	TM100010538	VAL100131145	Verification of compendia
Osmolality	Osmometry	TM100010540	VAL100131145	Verification of compendia
LNP Size and Polydispersity	Dynamic Light Scattering (DLS)	TM100010649	VAL100123376	Co-validation performed at Andover, MA and Chesterfield, MO
RNA Encapsulation and Content*	Fluorescence assay	TM100010402	VAL100123417	Co-validation performed at Andover, MA and Chesterfield, MO
RNA Integrity	Capillary Gel Electrophoresis	TM100010392	VAL100122020	Co-validation performed at Andover, MA and Chesterfield, MO
Lipid Content and Identity	HPLC-CAD	TM100010322	VAL100123165	Co-validation performed at Andover, MA and Chesterfield, MO
Container Content for injections	Volume of Injections in containers	TM100010614	VAL100131145	Verification of compendia
Identity of encoded RNA sequence	RT-PCR	TM100010407	VAL100121706	Co-validation performed at Andover, MA and Chesterfield, MO
In Vitro Expression	Cell-based flow cytometry	TM100010380	VAL100122803	Co-validation performed at Andover, MA and Chesterfield, MO
Bacterial Endotoxin	Endotoxin	Performed at DP manufacturing site	NA***	Kalamazoo, MI Puurs, Belgium NA
Sterility	Sterility	Performed at DP manufacturing site	NA***	Kalamazoo, MI Puurs, Belgium NA
CCI**	Dye Incursion	TM100010635	RPT-49795, INX100330679	Verification

*Also run as an IPC during DP manufacturing (INX100426408). Supplemental verification performed (VAL100121467)

**Run at T0 for stability batches only

***Verification results pending

(Note: Drug Product In-Process Controls, Sterility, and Endotoxin testing is performed at Pfizer Global Supply (PGS) Drug Product manufacturing sites)

Table 21. Validation Summary for the UV Spectroscopy Analytical Procedure (BioNTech)

Validation Parameter	Results
Precision – Repeatability (System)	RSD = ≤ 0.36%
Precision – Repeatability (Method)	RSD = ≤ 1.21%
Precision – Intermediate	RSD = ≤ 2.9%
Accuracy	99.0 – 101.1%
Specificity	Clear determination of the analyte in the presence of other substances.
Linearity	Linearity plot is linear by visual inspection Coefficient of determination (R^2) = 0.999
Range	Range (A260) = 0.2 – 1.0
Robustness (prepared sample stability, other)	The method is robust against different lots of cuvettes

Table 22. Validation Summary for the RT-PCR Analytical Procedure for Drug Substance (BioNTech)

Validation Parameter	Results
Specificity	The RT-PCR assay was able to detect RNA from BNT162b2 DS only and there was no cross reactivity with other non-specific RNA products. The positive signal was observed only in the positive control and the BNT162b2 DS samples. No positive signal was found in the non-target samples and negative control.
Robustness: Amplification extension time	The assay was able to detect RNA from DS using amplification extension times of 31, 33, and 35 seconds in the thermocycle profile.

Table 23. Validation Summary for the Capillary Gel Electrophoresis Analytical Procedure for Drug Substance (BioNTech)

Validation Parameter	Results
Precision – Repeatability (System)	RSD = 1.68 %
Precision – Repeatability (Method)	RSD = 0.64 %
Precision – Intermediate	RSD = 1.75 %
Accuracy	Recovery: 100 -102 %
Specificity	Specificity shown. No interference in the area of the RNA peak
Linearity (Concentration)	Linearity plot is linear by visual inspection in the range of 30 – 250 % Coefficient of determination (R^2) = 0.9832
Linearity (Integrity)	Linearity plot is linear by visual inspection in the range of 23.1 – 71.6 % Coefficient of determination (R^2) = 0.999

Table 24. Validation Summary for the qPCR Analytical Procedure (BioNTech)

Validation Parameter	Results
Precision – Repeatability (System)	RSD (level: Ct values) = ≤ 0.7% RSD (level: copies/mL) = ≤ 12.6%
Precision – Repeatability (Method)	RSD = 3.5%
Precision – Intermediate	RSD = ≤ 6.4%
Accuracy	98-101%
Specificity	Specificity is proven. Specific signal using RNA drug substance in comparison to a negative control sample.
Linearity	Linearity plot is linear by visual inspection Coefficient of determination (R^2) = 0.9974
Detection Limit (DL)	DL = 0.001 pg/mL
Quantitation Limit (QL)	QL = 0.1 pg/mL

Validation for the Bioburden Analytical Procedure (Rentschler)

The validation of the bioburden analytical procedure was performed following the principles described in USP <61>, Ph. Eur. 2.6.12, and JP 4.05 for BNT162b2 drug substance (DS). The method validation (challenge recovery test) challenges the test method to ensure that the test articles are non-inhibitory to the recovery of inoculated microorganisms.

The samples tested for the presence of bioburden are generally expected to be non-bacteriostatic/non-fungistatic, or to be readily neutralized by membrane filtration. To demonstrate this, the bioburden assay was performed with the addition of a microbial inoculum composed of specified organisms (USP recommended). The challenge recovery testing was performed for each test article with each microorganism along with the appropriate inoculum control.

The acceptance criterion for this validation was as follows:
In the presence of the test article, the mean count of each test organism may not differ by a factor greater than 2 from the value of the inoculum control.

Three batches of BNT162b2 DS (target concentration of 2.25 mg/mL), 10 mL in at least duplicates per organism, were tested for the validation of the method.

Table 25. Challenge Recovery Testing Results for Drug Substance (Rentschler)

Batch	Organism	Recovery factor
20E162001 (Rentschler 1071539)	*A. brasiliensis*	0.8
	B. subtilis	1.0
	C. albicans	0.8
	P. aeruginosa	1.0
	S. aureus	0.8
20E162002 (Rentschler 1071542)	*A. brasiliensis*	0.9
	B. subtilis	1.2
	C. albicans	0.9
	P. aeruginosa	1.3
	S. aureus	1.0
20E162003 (Rentschler 1071544)	*A. brasiliensis*	1.3
	B. subtilis	0.9
	C. albicans	1.6
	P. aeruginosa	1.3
	S. aureus	1.4

a. Challenge recovery testing was performed for each test article with each microorganism, as well as corresponding inoculum control in triplicate.

Validation for the Bacterial Endotoxin Analytical Procedure (Rentschler)

The validation of the bacterial endotoxins test, using the kinetic turbidimetric limulus amebocyte lysate (LAL) analytical procedure, for BNT162b2 drug substance (DS) was performed in alignment with the USP <85>, Ph. Eur. 2.6.14 and JP 4.01. The validation focused on the following parameters:

a. The criteria for the standard curve must be valid.
b. The sample solution must not interfere with the test (e.g. inhibition/enhancement)
c. The sample must have a maximum valid dilution (MVD) established.

The MVD is the maximum allowable dilution of the test article at which the endotoxin limit can be determined. The general equation to determine MVD is:

d. MVD = (endotoxin limit)/ (λ)
   Where,
   λ = 0.01 EU/mL (label sensitivity of the lysate)

Three batches of BNT162b2 DS (target concentration, 2.25 mg/mL) were diluted and tested. The inhibition/enhancement acceptance criterion for the spike recovery is 50-200%.

Table 26. Inhibition/Enhancement Results for BNT162b2 Drug Substance (Rentschler)

Batch Number	Sample Dilution	Spike Recovery (%)	Results (EU/mL)
20E162001 (Rentschler 1071539)	1:100	101	0.5049
		100	0.4985
		110	0.5487
20E162002 (Rentschler 1071542)	1:100	121	0.6079
		113	0.5636
		130	0.6506
20E162003 (Rentschler 1071544)	1:100	115	0.5775
		108	0.5387
		100	0.4979

Literature References

None

SUPPORTING DOCUMENTATION

New or Replaced Supporting Documentation

None

Previously submitted supporting documentation

Section S.4.3 Validation of Analytical Procedures (modRNA), SN0137

QUERY 19

Please provide the SOP and validation report for the in vitro expression assay for DP. With continued manufacturing experience, please update the stringency of the specification according to manufacturing process and assay capabilities.

RESPONSE 19

The SOP and validation report for the in vitro expression assay for the drug product are provided with this response. The sponsor acknowledges CBER's request to re-evaluate and update, if necessary, the stringency of the specification according to manufacturing process and assay capabilities.

Literature References

None

SUPPORTING DOCUMENTATION

New or Replaced Supporting Documentation

Test Method: TM1000010380, new

Validation Report: VAL100122803, new

Previously submitted supporting documentation

None

QUERY 20

Please note, all analytical assays must be validated prior to BLA submission. Complete validation reports should be submitted to the IND when they are available.

RESPONSE 20

Current validation data have been provided in the IND. The sponsor acknowledges that additional validation data should be provided following transfers of the release and stability methods to the additional commercial testing labs. After these transfers are completed, updated validation reports will be provided.

Literature References

None

SUPPORTING DOCUMENTATION

None