Analytical Method Transfer Exercise (AMTE) Report for TM100010392 Fragment Analyzer to test Drug Substance PF-07305885 From [redacted]

Test Method TM100010392 RNA-Integrity of mRNA Drug Substance and LNP-mRNA Drug Product Samples By Fragment Analyzer (CGE)

AMTE Protocol Number
GDMS: INX100453315 version 2.0

AMTE Report Number
GDMS INX100475657

APPROVAL

The approval of this document may be performed with an electronic signature using Pfizer Global Document Management System (GDMS) or a hand-written signature is acceptable when GDMS access is not available.

Pfizer 4.2 1st ind [redacted] **Transferring Laboratory (TL)**	
4.1(b) [redacted] ARD-Validation and Analytical Technology Transfer (VATT)	Document Approved Electronically
4.1(b) [redacted] (Data Verification (ARD-VATT)	Document Approved Electronically
4.1(b) [redacted] ARD-VATT	Document Approved Electronically
Pfizer 4.2 1st ind [redacted] **Receiving Laboratory (RL)**	
4.1(b) [redacted]	Document Approved Electronically
4.1(b) [redacted] Quality Assurance	Document Approved Electronically

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. Objective of Transfer

This analytical method transfer exercise (AMTE) report INX100475657 details the transfer of analytical method TM100010392 version (v) 6.0 "RNA integrity of mRNA drug substance and LNP-mRNA drug product samples by fragment analyzer" used for testing of PF-07305885 Drug Substance (DS) as per PLAN-8369, "Analytical method transfer plan for PF-07305885 (Covid Vaccine) Drug Substance to [redacted]." The successful completion of the procedures defined in protocol INX100453315 v2.0 provides assurance that the receiving lab can perform the test method. The Gap analysis and Risk Assessment for the Transfer of RNA integrity determination of BNT-162 (Covid-19 Vaccine) drug substance by fragment analyzer (TM100010392) from [redacted] is detailed in document ASMT-62863, Gap assessment for RNA Integrity by Capillary Gel Electrophoresis for BNT162b2 Drug Substance.

The method transfer was executed according to AMTE Protocol INX100453315 version 2.0 using the validated test method TM100010392 version 6.0, refer to validation report VAL100136603. After the successful completion of the AMTE, [redacted] will create a local version of the method which is based on TM100010392 v6.0 and was attached to the protocol. [redacted] method scope includes both drug substance and drug product. Since [redacted] will only be testing PF-07305885 DS, the scope of this AMTE only included the DS portion of TM100010392. Upon completion of the AMTE, the receiving lab will use their own method in the [redacted] documentation system that will only include the DS in scope of the method.

2. Testing Labs and Responsibilities

A list of testing laboratories and corresponding responsibilities are listed in Table 1.

Table 1: Laboratories and Corresponding Responsibilities

Lab	Responsibilities
[redacted]	

3. Mode of Transfer

The approach used in this AMTE was comparative testing where by the results generated by the RL were independently compared to validation data generated by the TL. The samples tested by the receiving lab were a subset of the samples that were designated for the validation of the method and documented in VAL100136603, hence no executions were performed at TL.

4. Equipment

To ensure that the laboratory instruments and equipment necessary to perform the test method were available and suitable for use the instruments used for the execution were qualified by completion of an installation, operation, and performance qualification (IQ/OQ/PQ). The instruments remained in a qualified state for the duration of the protocol execution period. The execution of the INX100453315 was used as a PQ of the instrument at the RL.

Table 2: Instrument and software for Receiving Laboratory

Lab	Instrument
4.2 1st ind	4.2 1st ind

The receiving lab was also required to obtain all the other necessary equipment, materials and reagents listed in the analytical test method TM100010392.

5. Training

4.2 1st ind has provided the necessary support in order for trained analysts to execute the protocol.

INX100475651 Training Memo for CGE AMTE INX100453315 to 4.2 1st ind, documents the virtual training the analyst received from Subject Matter Expert (4.1(b)) and a summary of the feasibility runs performed prior to execution of INX100453315 v2.0.

The successful completion of the transfer provides documented evidence that the analysts who executed the protocol are trained on TM100010392 v6.0.

6. Test Method Summary

TM100010392 v6.0 "RNA integrity of mRNA drug substance and LNP-mRNA drug product samples by fragment analyzer." This laboratory method describes the procedure used to determine the percent integrity of messenger RNA (mRNA), specifically nucleoside-modified RNA (modRNA) for BNT162. This procedure also applies to RNAs that are isolated from BNT162 lipid nanoparticle (LNP) DPs by disruption of the nanoparticles. The method was validated and documented in validation report VAL100136603. The Fragment Analyzer system is a multiplexed capillary electrophoresis (CE) instrument for performing automated, high throughput separation and quantification of mRNA. Separation is achieved by applying an electric field through a narrow bore (50 µm i.d.) fused silica capillary array filled with a conductive gel matrix designed to sieve RNA molecules of a specific size range. When a high voltage is applied to the capillary array, injected RNA migrates through the gel matrix as a function of length or size, with smaller sized fragments eluting faster than larger sized

fragments. At a point toward the far end of the capillary array, detection of the separated RNA is achieved by fluorescence of a sensitive intercalating dye present in the separation gel matrix, which fluoresces when bound to RNA molecules. The Fragment Analyzer system utilizes a high intensity light emitting diode (LED) excitation light source that is focused across the capillary array detection window and imaged onto a sensitive, two-dimensional charge coupled device (CCD) detector. By monitoring the relative fluorescence unit (RFU) intensity as a function of time during the CE separation, digital electropherograms representative of the RNA content of 48 samples are collected in a single experimental run.

7. Observations/Deviations/Events

4.2 1st ind [redacted] For additional details see GDMS Document INX100483867 Investigation to Change Acceptance Criteria for AMTE Protocol INX100453315 v2.0

8. AMTE Procedure (Precision)

10.1. Analysis of Drug Substance

The assessment of Precision for drug substance was performed by evaluating varying preparations of Samples B, D and E as described in TM100010392.

10.2. Testing Plan

The samples were tested across a total of 2 instances. The analysis was performed by two analysts in 4.2 1st ind [redacted] (RL) on one instrument according to the testing plan shown in **Table 3**. For the purpose of evaluating method capabilities, the % RNA Integrity was displayed to one decimal place even if present below method LOQ. Due to the low values observed for precision elements of the validation the %RSD was also expressed with one decimal to demonstrate the method capabilities. The acceptance criteria were expressed as whole numbers to align with the method and product specifications. The AMTE Coordinator calculated the precision for drug substance expressed

as the overall %RSD derived from the normalized results (n=18) across all three samples (B, D, and E).

Table 3: AMTE Samples Precision-Experimental Design – [redacted]

Instance	Analyst	Instrument	Preparations **DS:**		
			Sample B	Sample D	Sample E
1	1	1	3	3	3
2	2	1	3	3	3

For the purpose of this plan, an instance is a separate experiment performed by the designated analyst using the designated equipment. Each sample was prepared three times and measured in triplicate per the method.

No alterations/substitutions were made during the execution of the transfer protocol.

The TL [redacted] results were acquired through the validation activities (VAL100136603 v2.0). The RL acquired data following the test matrix in **Table 3**.

10.3. Sample Analysis

The sample analysis was performed as described per the test method. For each determination, the analysts recorded the reportable result as the average RNA Integrity (%) with one decimal.

10.4. Documentation

Documentation of the instances (each experiment) were completed per the current site process. The reviewed data was compiled and sent to the protocol coordinator/author for authoring of the report. The AMTE coordinator processed the RL data in the calculation workbook INX100475656, and the RL data in the workbook was verified by the RL. The AMTE report contains RL data compared to the results of the validation documented in VAL100136603 v2.0.

9. AMTE Samples

The analysts were provided drug substance Reference Material 20Y513C701-WRM and the pre-prepared AMTE test samples.

The samples utilized in this AMTE were a subset of the samples that were designated for the validation of the method documented in the validation report (VAL100136603 v2.0). Validation DS samples B, D, and E were analyzed by the analysts per TM100010392 v6.0. The details of the sample designation, sample description and material identification can be found in **Table 4**. The samples were a series of spiked samples containing different levels (RNA Integrity (%)) that were prepared prior to the validation of the method. The samples listed in **Table 4** were tested and evaluated with the results obtained from the validation documented in VAL100136603 v2.0 for only DS Samples.

Table 4: AMTE Samples

Sample Designation	Sample Description	Material ID	Concentration (mg/mL)	Shipping Temperature (°C)	Storage Temperature (°C)
B	Drug Substance at 4.2 mRNA Integrity (spiked)	00713785-0152-M02	4.2 1st ind		
D	Drug Substance at 4.2 mRNA Integrity (spiked)	00713785-0152-M04			
E	Drug Substance at 4.2 mRNA Integrity (spiked)	00713785-0152-M05			

10. System Suitability (Assay Acceptance Criteria)

The assay acceptance criteria, as described in the method, was met for the data incorporated into the AMTE results.

11. AMTE Data Evaluation

The analysts calculated final RNA Integrity (%) for Samples B, D, and E per test method and reported to one decimal place.

AMTE Coordinator:

- Workbook: Calculated and reported the mean, standard deviation, RSD (%) and 4.2 1st ind confidence interval at each sample level (reported to one decimal place).
- Determined if the average RNA Integrity (%) from each sample is within 4.2 times the standard deviation of the average value for each respective sample value documented in the reproducibility section (see Deviation 1) of the validation report VAL100136603 v2.0.
- Workbook: Calculated the overall mean, overall standard deviation, overall 4.2 confidence interval and overall %RSD (n=18) from the individual normalized values obtained by dividing each reportable by the average RNA Integrity (%) of each respective level. Report the overall %RSD to one decimal observed as the Precision.
- Workbook/Report: Evaluated the results against the corresponding acceptance criteria.

12. AMTE Acceptance Criteria

The receiving lab demonstrated acceptable precision and generated results that are comparable to the transferring lab. The RL data was compared to the TL data for lab to lab comparability. Table 5 details the acceptance criteria for lab-to-lab comparability and precision.

Table 5: AMTE Corrected Acceptance Criteria (See Deviation 1)

Parameter	Acceptance Criteria
Comparability between TL and RL	The average RNA Integrity (%) of the RL should be within 4.2 1st the standard deviation of the average RNA Integrity (%) for each sample of the reproducibility - precision of the TL documented in VAL100136603 v2.0 for Samples B, D, and E.
RL Precision for RNA Integrity (%)	Overall RSD (%) 4.2 for Samples B, D, and E.

Sample	Average (%)	SD	4.2 x SD
B	4.2 1st ind		
D			
E			

13. Results and Conclusion

The comparability between the average RNA Integrity (%) of the RL was determined to be within 4.2 1st ind RNA Integrity (%) for each sample of the reproducibility - precision of the TL documented in VAL100136603 for Samples B, D, and E. See Table 6 for a summary of the results from calculation workbook INX100475656 along with Figure 1, Figure 2, and Figure 3. The RL Precision for RNA Integrity (%) was determined to be within the acceptance criteria. Based on the scope of the study performed (across four labs, eight instruments, and seven analysts), the level of variability observed is considered acceptable and a practical representation of method capability and performance. See the results summarized in Table 7. See also Attachment 1, Attachment 2, and Attachment 3.

Table 6 Comparability between TL and RL

Sample	Average from Validation	SD from Validation	4.2 x SD from Validation	Minimum Acceptable Value	Maximum Acceptable Value	Result from RL	Pass/Fail
B	4.2 1st ind						Pass
D							Pass
E							Pass

Figure 1 AMTE Acceptance Criterion Sample B

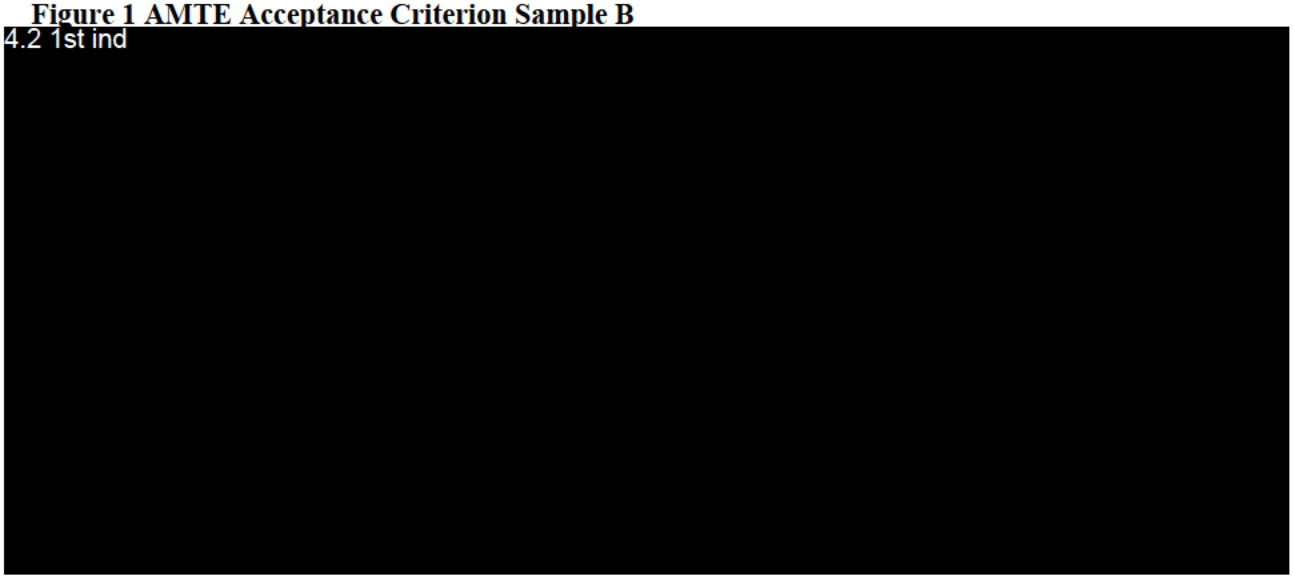

Figure 2 AMTE Acceptance Criterion Sample D

Figure 3 AMTE Acceptance Criterion Sample E

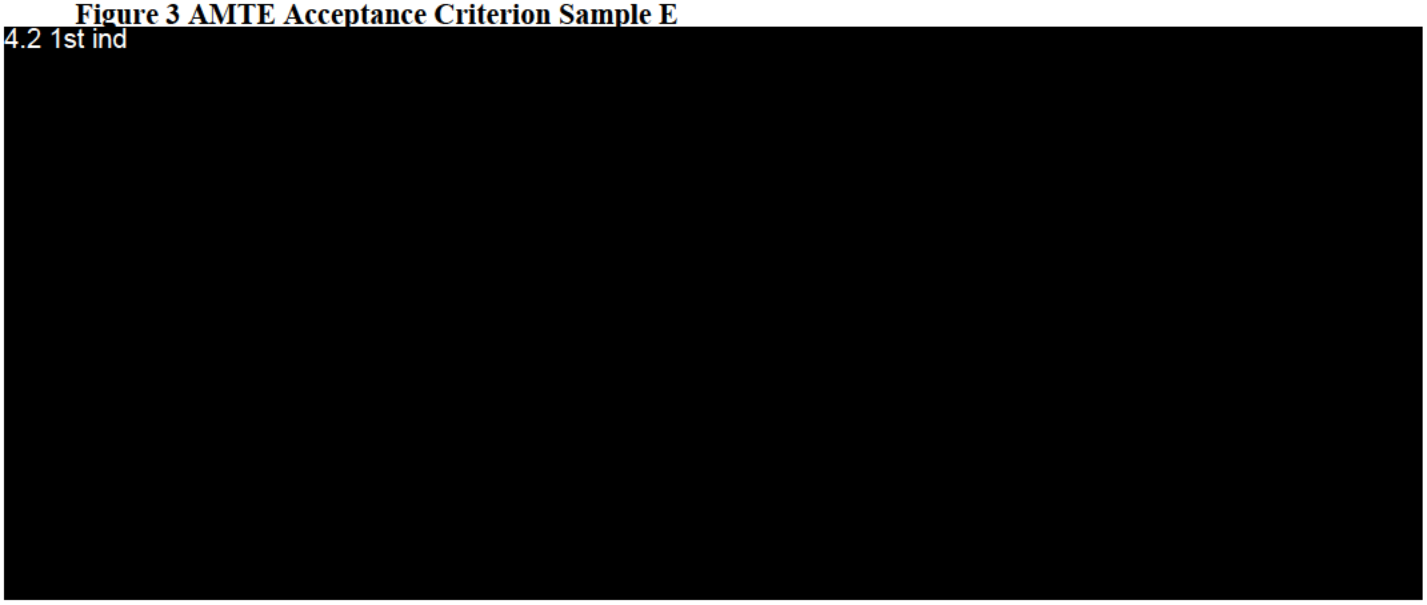

Table 7 RL Precision for RNA Integrity (%)

Sample Designation	Sample Description	Acceptance Criteria	%RSD	Criteria Evaluation
B	DS 4.2 mRNA Integrity	Normalized %RSD 4.2	4.	Pass
D	DS 4.2 mRNA Integrity			
E	DS 4.2 mRNA Integrity			

The successful completion of the procedures defined in this report provides assurance that the receiving lab, 4.2 1st ind can perform the test method.

All AMTE acceptance criteria for comparability and precision were met, demonstrating that the RL is capable of performing the method for its intended use. The 4.2 1st ind is therefore deemed qualified to execute the method for GMP purposes.

14. References

14.1 GDMS Document INX100432885 v2.0: Analytical Transfer Plan for PF-07302048 (Covid-19 Vaccine) from 4.2 1st ind

14.2 GDMS document: Test Method TM100010392 v6.0 (attached) RNA Integrity of mRNA Drug Substance and LNP-mRNA Drug Product Samples by Fragment Analyzer.

14.3 GDMS document VAL100136603 v2.0: Validation Report for Protocol VAL100130942 TM100010392 Determination of RNA Integrity of mRNA Drug Substance and LNP-mRNA Drug Product Samples by Fragment Analyzer

14.4 GDMS document: INX100421728 v11.0 Specification Report for PF-07305885 COVID-19 Vaccine BNT162b2 mRNA Drug Substance.

14.5 GMDS document: SOP-LAB-01246 v4.0, [redacted] Analytical Method Transfer Exercise Processes.

14.6 ICH Q2 (R1). Validation of Analytical Procedures: Text and Methodology. November 2005

14.7 ICH Q6B. Specifications: Test procedure and acceptance criteria for biotechnological and biological products. 1999.

14.8 SOP-13362 v22.0 Method Development, Qualification, Transfer and Validation.

14.9 SOP-13147 v20.0 Verification of Analytical Methods.

14.10 PLAN-8369 v3.0 Analytical method transfer plan for PF-0730588 (Covid Vaccine) Drug Substance to [redacted]

14.11 ASMT-62863 V1.0() Gap assessment for RNA Integrity by Capillary Gel Electrophoresis for BNT162b2 Drug Substance.

14.12 GDMS document: INX100475656 Calculation Workbook for AMTE INX100475656.

15. Revision History

Version	Change	Justification for Change
1.0	New Document	New report to transfer analytical method TM100010392 [redacted]

16. Attachments

Attachment 1 AMTE DS Precision Summary

Sample Designation	Sample Description	Acceptance Criteria	%RSD	Criteria Evaluation
B	DS 4.2 mRNA Integrity	Normalized %RSD 4.2	4.	Pass
D	DS 4.2 mRNA Integrity			
E	DS 4.2 mRNA Integrity			
LAB 4.2 1st ind				
Instance	**Analyst**	**Instrument**	**Sample Prep (Samples B, D, and E)**	
1	1	1	3	
2	2	1	3	

Attachment 2 RNA Integrity Samples B, D, and E

Sample B = 4.2 1st ind mRNA Integrity

Instance	Prep 1	Prep 2	Prep 3	Mean	SD	%RSD	4.2 CI
1 (INX100453315EF031121_011)	4.2 1st ind						
2 (INX100453315SJ031121_013)							
Instance 1-2 (n= 6)				4.2 1st ind			

Sample D = 4.2 1st ind mRNA Integrity

Instance	Prep 1	Prep 2	Prep 3	Mean	SD	%RSD	4.2 CI
1	4.2 1st ind						
2							
Instance 1-2 (n= 6)				4.2 1st ind			

Sample E = 4.2 1st ind mRNA Integrity

Instance	Prep 1	Prep 2	Prep 3	Mean	SD	%RSD	4.2 CI
1	4.2 1st ind						
2							
Instance 1-2 (n= 6)				4.2 1st ind			

Attachment 3 Normalized RNA Integrity Values for Samples B, D, and E

Instance/Samples (B, D, and E)	Normalized RNA Integrity (%) P1	Normalized RNA Integrity (%) P2	Normalized RNA Integrity (%) P3
1/B	4.2 1st ind [redacted]		
2/B			
1/D			
2/D			
1/E			
2/E			
Normalized Mean (n= 18)	4.2 1st ind [redacted]		
SD (n=18)			
%RSD (n=18)			
4.2 1st ind **CI (n=18)**			

Document Approval Record

Document Name:	INX100475657
Document Title:	Analytical Method Transfer Exercise (AMTE) Report for TM100010392 Fragment Analyzer to Test Drug Substance PF-0705885 From ARD to 4.2 1st ind

4.1(b)

[redacted]