SUMMARY OF ANALYTICAL METHOD TRANSFER FOR BNT162B2 DRUG PRODUCT RELEASE AND STABILITY METHODS

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1. BACKGROUND

Pfizer and BioNTech are developing BNT162b2, an RNA-based vaccine against SARS-CoV-2 coronavirus. The development efforts are global and require work sharing among partners. The analytical testing strategy for drug product has evolved rapidly throughout development as drug product testing sites have changed.

At Polymun, the release and stability testing of clinical trial material for phase 1, 2 and 3 has been performed with analytical platform methods. In parallel, product-specific methods have been developed and optimized by Pfizer Analytical Research & Development laboratories (Andover and Chesterfield, USA). This has included the introduction of new methods such as the potency assay based on in vitro expression determined by cell-based flow cytometry.

All methods have been validated in Pfizer Analytical Research & Development laboratories to support clinical and commercial drug product (DP) release and stability testing. Pfizer laboratories will be used initially to test drug product in most markets globally. Recently, the laboratories in Andover and Chesterfield have started to transfer methods to EU test sites.

2. OBJECTIVE

The purpose of this document is to summarize the analytical method transfer strategy for all testing used to support routine release and stability of the BNT162b2 (SARS-CoV-2 Vaccine) Drug Product (DP).

3. GENERAL INFORMATION

3.1. Testing Sites

The table below shows the network of release testing sites and releasing entities at the time of product launch and as it is presented in the MAA in CTD Section 3.2.P.3.1.

Testing step	Testing facility	Alternate testing sites
DP release testing: Sterility	Pfizer Manufacturing Belgium NV, Rijksweg 12, 2870 Puurs, Belgium	Hospira Zagreb Ltd. Prudnička cesta 60 10291 Prigorje Brdovečko Croatia or SGS Lab Simon SA Vieux Chemin du Poète 10 Wavre, 1301 Belgium
DP release testing: Endotoxin, Composition and Strength	Pfizer Manufacturing Belgium NV, Rijksweg 12, 2870 Puurs, Belgium (Pfizer Puurs)	
DP release testing: Identity and Subvisible particles	Pfizer Ireland Pharmaceuticals, Grange Castle Business Park, Clondalkin, Dublin 22, Ireland (Pfizer Grange Castle)	
DP release testing: Composition and Strength, Identity, Potency, Purity and Adventitious Agents	Wyeth BioPharma, Division of Wyeth Pharmaceuticals LLC, 1 Burtt Road, Andover, MA 01810, USA	Pfizer Inc., 875 Chesterfield Parkway West, Chesterfield, MO 63017, USA
Final release	BioNTech Manufacturing GmbH Kupferbergterrasse 17 - 19 55116 Mainz, Germany	Pfizer Manufacturing Belgium NV, Rijksweg 12, 2870 Puurs, Belgium

Analytical test methods are being transferred from Pfizer Analytical Research and Development laboratories located in Andover, MA and Chesterfield, MO (Transferring Laboratory, TL) to Pfizer labs in Puurs, Belgium (Receiving Laboratory, RL1) and Grange Castle, Ireland (Receiving Laboratory, RL2).

3.2. Product Description

BNT162b2 is a vaccine candidate for the prevention of COVID-19. The vaccine uses mRNA that encodes for a spike protein of the SARS-CoV-2 (novel corona virus). The drug product is a preservative-free, sterile dispersion of mRNA formulated in lipid nanoparticles (LNP) in aqueous cryoprotectant buffer for intramuscular administration.

3.3. Summary of Manufacturing

BNT162b2 is a vaccine candidate that is manufactured by controlled mixing of mRNA and lipids in a solvent environment conductive to the formation of nanoparticles. A mRNA stock solution is prepared by dilution of the mRNA drug substance in citrate buffer. Separately, a stock solution of lipids ALC-0315, ALC-0159, DSPC, and cholesterol are diluted in ethanol and is filtered through a 0.2 µm filter. The lipid and mRNA solutions are in-line mixed at a controlled rate to form the LNP followed by an in-line co-dilution with citrate buffer. Next, the diluted bulk drug substance is subjected to concentration, buffer exchange, and filtration steps. The bulk drug product then is diluted to the final concentration with PBS buffer and a cryoprotectant (sucrose) solution. The bulk drug product is then sterile filtered and is aseptically filled into sterile glass vials, sealed and stored at -90°C to -60°C.

3.4. Testing summary and in-scope methods

This document summarizes the transfer of routine release, and stability testing from the TL to the respective RLs. Table 1 includes a summary of the in-scope routine testing, the mode of transfer and the corresponding site performing the testing.

Table 1. Summary of Testing, Mode of Transfer and Sites Performing Testing

Quality Attribute	Analytical Procedure	Test Purpose	Mode of Transfer	Testing Lab	
				Puurs (RL1)	Grange Castle (RL2)
Composition and Strength					
Appearance	Appearance (Visual)	Release/Stability	Local Verification (Compendial)	X	
Appearance (Visible Particulates)	Appearance (Particles)	Release/Stability	Local Verification (Compendial)	X	
Subvisible particles	Subvisible particulate matter	Release/Stability	Local Verification (Compendial)	X	X
pH	Potentiometry	Release/Stability	Local Verification (Compendial)	X	
Osmolality	Osmometry	Release	Local Verification (Compendial)	X	
Container content for injections	Extractable Volume	Release	Local Verification (Compendial)	X	
LNP Size and Polydispersity	Dynamic Light Scattering (DLS)	Release/Stability	Comparative Testing	X	
RNA Encapsulation and Content	Fluorescence assay	Release/Stability	Co-Validation	X	
ALC-0315 Content	HPLC-CAD	Release/Stability	Comparative Testing	X	
ALC-0159 Content	HPLC-CAD	Release/Stability	Comparative Testing	X	
DSPC Content	HPLC-CAD	Release/Stability	Comparative Testing	X	
Cholesterol Content	HPLC-CAD	Release/Stability	Comparative Testing	X	

Table 1. Summary of Testing, Mode of Transfer and Sites Performing Testing

Quality Attribute	Analytical Procedure	Test Purpose	Mode of Transfer	Testing Lab	
				Puurs (RL1)	Grange Castle (RL2)
Potency					
In Vitro Expression	Cell-based flow cytometry	Release/Stability	Co-Validation		X
Identity					
Identity of encoded RNA sequence	RT-PCR	Receipt ID, Release	Co-Validation		X
Lipid Identities (in DP)	HPLC-CAD	Release	Comparative Testing	X	
Purity					
RNA Integrity	Capillary Gel Electrophoresis	Release/Stability	Comparative Testing	X	
Adventitious Agents					
Bacterial Endotoxins	Endotoxin (LAL)	Release/Stability	Local Verification (Compendial)	X	
Sterility	Sterility	Release/Stability	Local Verification (Compendial)	X	
Container Closure Integrity	Dye incursion	Stability	Local Verification (Compendial)	X	

3.5. Transfer Strategy

Method transfer is performed by local verification testing, co-validation, or analytical method transfer exercise (AMTE)-comparative testing.

For transfer by co-validation, the test method will be co-validated by the TL and RL to demonstrate suitability for its intended use. The co-validation with ARD, PGS Puurs or PGS Grange Castle will utilize appropriate samples identified by the TL. The co-validation will serve as the transfer process to all participating sites to ensure that the RLs have the proper equipment and technical capabilities to routinely perform the analytical method detailed within the protocol and can achieve results comparable to those of the TL.

For transfer for by local validation, the test methods are classified as compendial and/or the receiving laboratory has previous experience in performing these methodologies for other products. Since these tests are general compendial or in-house compendial based tests that are routinely performed at each site, verification per site procedure must be performed by the site to document that the methods can be successfully executed for the test articles under typical conditions of use.

For transfer by comparative testing, the test method will be validated at the TL to demonstrate suitability for its intended use and then transferred to RL1 (PGS Puurs) or RL2 (PGS Grange Castle). RL1 or RL2 will perform comparative testing using appropriate samples and the data will be compared to the validation or other suitable data. The comparative testing performed at RL1 or RL2 will serve as the transfer process to the site to ensure that the RL has the proper equipment and technical capabilities to routinely perform the analytical method detailed within the protocol and can achieve results comparable to those of the TL.

4. TRAINING

Method specific training will be provided by the TL in advance of or by the transfer activities. Technology specific training may occur prior to initiation of transfer activities. Upon completion of the transfer, analysts are considered trained in the methods. In some instances, the receiving laboratory has experience in performing methods in scope of the transfer. Therefore, no additional training is required, with exception of current general training requirements per site procedures for the specified methods.

5. TRANSFER SCHEDULE

Analytical method transfer is conducted in a stepwise manner to ensure the exercise is performed in a compliant and well-controlled manner. Method transfer includes the following steps:

a. Preparation of RL laboratories and qualification of requisite instrument and equipment.

b. Creation of transfer or compendial verification protocol.

c. Training and feasibility testing between TL and RL, where required.

d. Determination of transfer acceptance criteria.

e. Preparation of transfer samples/materials.

f. Approval of transfer protocol.

g. Execution of transfer testing by TL and RL, where required.

h. Data review and compilation

i. Creation of transfer or compendial verification report

j. Implementation of testing at RL

The drug product testing sites and transfer schedules are provided in **Table 2.** Transfer of the compendial tests and drug product identity test will be completed by Dec 31, 2020 as the EU receiving laboratory has experience in performing these test methods. The remaining tests transferred by co-validation or comparative testing will be completed by May 2021 (In Vitro Expression) and August 2021 (all other tests) based on the time required to complete the aforementioned steps.

Table 2. Drug Product Release Testing Sites and Transfer Schedule

Quality Attribute	Analytical Procedure	Drug Product Release Testing following Initial MAA Approval	Transfer to EU site completed by	Receiving Laboratory (EU Lab)
Composition and Strength				
Appearance	Appearance (Visual)	Pfizer Puurs	31 Dec 2020	n/a
Appearance (Visible Particulates)	Appearance (Particles)	Pfizer Puurs	31 Dec 2020	n/a
Subvisible particles	Subvisible particulate matter	Pfizer Grange Castle	31 Dec 2020	n/a
pH	Potentiometry	Pfizer Puurs	31 Dec 2020	n/a
Osmolality	Osmometry	Pfizer Puurs	31 Dec 2020	n/a
Container content for injections	Volume of injections in containers	Pfizer Puurs	31 Dec 2020	n/a
LNP Size	Dynamic Light Scattering (DLS)	Pfizer Andover & Pfizer Chesterfield	31 Aug 2021	Pfizer Puurs
LNP Polydispersity	Dynamic Light Scattering (DLS)	Pfizer Andover & Pfizer Chesterfield	31 Aug 2021	Pfizer Puurs
RNA Encapsulation	Fluorescence assay	Pfizer Andover & Pfizer Chesterfield	31 Aug 2021	Pfizer Puurs
RNA Content	Fluorescence assay	Pfizer Andover & Pfizer Chesterfield	31 Aug 2021	Pfizer Puurs

ALC-0315 content	HPLC-CAD	Pfizer Andover & Pfizer Chesterfield	31 Aug 2021	Pfizer Puurs
ALC-0159 content	HPLC-CAD	Pfizer Andover & Pfizer Chesterfield	31 Aug 2021	Pfizer Puurs
DSPC content	HPLC-CAD	Pfizer Andover & Pfizer Chesterfield	31 Aug 2021	Pfizer Puurs
Cholesterol content	HPLC-CAD	Pfizer Andover & Pfizer Chesterfield	31 Aug 2021	Pfizer Puurs
Potency				
In Vitro Expression	Cell-based Flow Cytometry	Pfizer Andover & Pfizer Chesterfield	31 May 2021	Pfizer Grange Castle
Identity				
Lipid identities	HPLC-CAD	Pfizer Andover & Pfizer Chesterfield	31 Aug 2021	Pfizer Puurs
Identity of encoded RNA sequence	RT-PCR	Pfizer Grange Castle	31 Dec 2020	n/a
Purity				
RNA Integrity	Capillary Gel Electrophoresis	Pfizer Andover & Pfizer Chesterfield	31 Aug 2021	Pfizer Puurs
Adventitious Agents				
Bacterial Endotoxins	Endotoxin (LAL)	Pfizer Puurs	Completed prior Dec	n/a
Sterility	Sterility	Pfizer Puurs, Hospira Zagreb, SGS Wavre	Completed prior Dec	n/a
Container Closure Integrity	Dye incursion	Pfizer Andover & Pfizer Chesterfield	31 Aug 2021	Pfizer Puurs

6. CONCLUSIONS

Upon successful completion of all transfer testing (co-validation, AMTE, or local verification), drug product release and stability testing will be performed on EU soil. If amendments to this plan are required, these changes will be captured in the final report.

7. GLOSSARY

- ARD: Analytical Research and Development
- GC: Grange Castle
- PGS: Pfizer Global Supply
- GDMS: Global Document Management System
- LNP: lipid nanoparticles
- TL: Transferring Laboratory
- RL: Receiving Laboratory
- AMTE: Analytical Method Transfer Exercise
- N/A: Not Applicable
- DP: Drug Product
- DSPC: 1,2-Distearoyl-sn-glycero-3-phosphocholine

8. DOCUMENT HISTORY

Version	Summary of changes	Justification of change
Version 1.0	New document	Initial version

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