3.2.S.2.6. ANALYTICAL METHOD EVOLUTION

The analytical testing strategy applied to BNT162b2 drug substance has evolved throughout the development history. These changes to the analytical testing strategy are summarized in Table 3.2.S.2.6-1.

Table 3.2.S.2.6-1. Evolution of BNT162b2 Drug Substance Methods

Process		Clinical (Process 1)	Emergency Supply[a] (Process 2)	Process Performance Qualification, Commercial Supply (Process 2)
Quality Attribute	**Analytical Procedure**			
Clarity	Appearance	R	R	R, S
Coloration	Appearance	R	R	R, S
pH	Potentiometry	R	R	R, S
Osmolality	Osmometry	R	Not required	Not required
RNA sequence	Sequencing of DNA starting material	R	Not required	Not required
Content (RNA concentration)	UV Spectroscopy	R, S	R, S	R, S
Identity: RNA length	Agarose gel electrophoresis	R	Not required	Not required
Identity: as RNA		R	Not required	Not required
Identity of encoded RNA sequence	RT-PCR	Not required	R	R
RNA integrity	Capillary gel electrophoresis	R, S	R, S	R, S
5'-Cap	HPLC-UV	Not required	Not required	R, S
Poly(A) tail	ddPCR	Not required	Not required	R, S
Residual DNA template	qPCR	R	R	R
Residual double stranded RNA (dsRNA)	Immunoblot	R	R	R
Bacterial endotoxins	Endotoxin (LAL)	R	R	R, S
Bioburden	Bioburden	R	R	R, S

a. Emergency supply designation applies to U.S. market.
Abbreviations: R = Release; S = Stability; RT-PCR = reverse transcription polymerase chain reaction; ddPCR = droplet digital PCR; qPCR = quantitative PCR; dsRNA = double stranded RNA; LAL = Limulus amebocyte lysate

3.2.S.2.6.1. Description of Changes and Method Bridging

Description of method changes and results of appropriate bridging experiments are detailed in Sections 3.2.S.2.6.1.1 to 3.2.S.2.6.1.4. For some methods both the historic and new methods were tested concurrently, and the data compared.

3.2.S.2.6.1.1. Osmolality

Osmolality was employed as a surrogate for monitoring levels of ethanol and NaCl following the magnetic bead purification step in process 1. With the implementation of process 2, and

its associated purification steps, this analytical control is no longer required at the drug substance stage.

3.2.S.2.6.1.2. RNA Sequence by Sequencing of DNA Starting Material

Testing of the DNA starting material (process 1) to ensure the correct DNA template for transcription has been removed from the BNT162b2 drug substance release testing. With the change from DNA template to plasmid DNA, concomitant with the change from process 1 to process 2, the analytical control of the plasmid DNA sequence integrity occurs during plasmid release testing as described in Section 3.2.S.2.3. Source, History and Generation of Plasmids.

3.2.S.2.6.1.3. Agarose Gel Electrophoresis and RT-PCR

Agarose gel electrophoresis was originally positioned as an identity method for BNT162b2 drug substance. Denatured RNA samples were separated by denaturing gel electrophoresis on a precast and buffered agarose gel pre-stained with a nucleic-acid specific dye. The gel was photographed with a gel documentation system. The length of the RNA band was compared to an RNA of a known size length standard (RNA ladder). In addition, an RNA sample was incubated for a defined time period with RNAse A, certified to be free of DNases and proteases. It was then separated by gel-electrophoresis on a precast and pre-stained agarose gel and compared to an RNA sample that had been incubated under identical conditions except for the addition of RNase A. The disappearance of the RNA band upon incubation with RNase A verifies the identity as RNA. Results are reported as the presence or absence of an RNA band by gel electrophoresis.

The agarose gel electrophoresis method has been replaced with the reverse transcription-polymerase chain reaction (RT-PCR) method to confirm the encoded RNA sequence, as described in Section 3.2.S.4.2 Analytical Procedures – RT-PCR. This method positively identifies the encoded RNA sequence as BNT162b2, and demonstrates identity as RNA, as only RNA can be reverse transcribed.

Specificity data generated during the qualification of the RT-PCR method, as presented in Section 3.2.S.4.2 Validation of Analytical Methods – RT-PCR, demonstrate that the method is suitable for use as an identity method for BNT162b2 drug substance. In addition, four batches of drug substance were analyzed by both the Agarose Gel Electrophoresis and RT-PCR methods. All batches passed the respective acceptance criteria for identity for both methods, as detailed in 3.2.S.2.6 Developmental History and Comparability Assessment.

3.2.S.2.6.1.4. Capillary Gel Electrophoresis

The capillary gel electrophoresis method for RNA integrity was modified with respect to data processing. During method development the software for data evaluation was changed, resulting in lower absolute integrity numbers, which is mainly caused by different baseline subtraction settings. Raw data for three BNT162b2 drug substance batches were reprocessed with updated data processing parameters. The original and updated values appear in Table 3.2.S.2.6-2, alongside data generated during side-by side analytical comparability as detailed in 3.2.S.2.6 Developmental History and Comparability Assessment.

Table 3.2.S.2.6-2. Capillary Gel Electrophoresis - Comparison of Processing Methods

BNT162b2 Drug Substance Batch	RNA Integrity Original Processing Method (BNT)	RNA Integrity Updated Processing Method (BNT)	RNA Integrity Updated Processing Method (PFE, Comparability)
R427-P020.2-DS	4.2 1st ind.		
R438-P020.2-DS			
R443-P020.2-DS			