

2.3.S.4. CONTROL OF DRUG SUBSTANCE,OMICRON (B.1.1.529) VARIANT

This section contains information specific for presentation Tris/sucrose Comirnaty [Original and Omicron (B.1.1.529)], which is discontinued. For information purposes, data/information supportive of the platform development approach for other presentations is maintained.

2.3.S.4.2. Analytical Procedures

Analytical procedures for (B.1.1.529) Omicron variant drug substance (DS) release and stability testing are listed in Table 2.3.S.4-1. Several of the analytical procedures are identical to the corresponding commercial BNT162b2 original vaccine procedures, while others have been updated for analysis of the Omicron (B.1.1.529) variant. For identity testing, Omicron variant-specific reagents are utilized and the analytical procedure for assessment of identity. Additionally, the qPCR method description was updated to allow for an additional calculation which accounts for a base pair size difference between original and variant.

Table 2.3.S.4-1. Analytical Procedures for Omicron (B.1.1.529) Drug Substance

| Analytical Procedure | Quality Attribute | Original Vaccine Analytical Procedure Updated for Omicron Variant (Yes/No) |
|-------------------------------|----------------------------------|--|
| Appearance | Clarity and Color | No |
| Potentiometry | pH | No |
| UV Spectroscopy | Content (RNA Concentration) | No |
| RT-PCR | Identity of encoded RNA sequence | Yes |
| RP-HPLC | 5'-Cap | No |
| ddPCR | Poly (A) Tail | No |
| IP-RP-HPLC | Poly (A) Tail Length | No |
| qPCR | Residual DNA Template | Yes |
| Immunoblot | Residual dsRNA | No |
| Capillary Gel Electrophoresis | RNA Integrity | No |
| Endotoxin (LAL) | Bacterial Endotoxin | No |
| Bioburden | Bioburden | No |

Abbreviations: ddPCR = digital droplet polymerase chain reaction, dsRNA = double stranded RNA, LAL = limulus amoebocyte lysate, qPCR = quantitative polymerase chain reaction, RP-HPLC = reversed phase-high performance liquid chromatography, RT-PCR = reverse transcription-polymerase chain reaction

Analytical procedures for the control of Omicron (B.1.1.529) variant drug substance, including those common to BNT162b2 drug substance and BNT162b2 drug product, are listed in [Table 2.3.S.4-2](#) and [Table 2.3.S.4-3](#).

Table 2.3.S.4-2. Analytical Procedures Common to Omicron (B.1.1.529) Drug Substance and Omicron (B.1.1.529) Drug Product

| Analytical Procedure | Quality Attribute |
|-------------------------------|----------------------------------|
| Potentiometry | pH |
| RT-PCR | Identity of Encoded RNA Sequence |
| Capillary Gel Electrophoresis | RNA Integrity |

Abbreviations: RT-PCR = reverse transcription polymerase chain reaction

Table 2.3.S.4-3. Analytical Procedures for Omicron (B.1.1.529) Drug Substance Only

| Analytical Procedure | Quality Attribute |
|----------------------|-----------------------------|
| Appearance | Clarity and Coloration |
| UV Spectroscopy | Content (RNA Concentration) |
| RP-HPLC | 5'-Cap |
| ddPCR | Poly (A) Tail |
| IP-RP-HPLC | Poly(A) Tail Length |
| qPCR | Residual DNA Template |
| Immunoblot | Residual dsRNA |
| Endotoxin (LAL) | Bacterial Endotoxin |
| Bioburden | Bioburden |

Abbreviations: ddPCR = digital droplet polymerase chain reaction, IP-RP-HPLC = ion-pairing reversed phase-high performance liquid chromatography, dsRNA = double stranded RNA, qPCR = quantitative polymerase chain reaction, RP-HPLC = reversed phase-high performance liquid chromatography

2.3.S.4.3. Validation of Analytical Procedures

Validation of analytical procedures was performed to ensure the composition, strength, identity, purity, and safety of Omicron (B.1.1.529) variant drug substance. All non-compendial and compendial analytical procedures were confirmed suitable for their intended use.

The Omicron (B.1.1.529) variant differs from the original vaccine in the sequence and length of the mRNA. The DS concentration, formulation process, quality attributes, and process control remain unchanged in the Omicron (B.1.1.529) variant drug substance. Identical test methods used for the original drug substance are used for the Omicron (B.1.1.529) variant drug substance apart from the method for identity by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) which requires Omicron (B.1.1.529) variant specific reagents. The validation of RT-PCR is presented in Section 3.2.S.4.3 Validation of Analytical Procedures – RT-PCR [Omicron B.1.1.529 Variant].

The remainder of the DS analytical procedures are not impacted by the change to the mRNA sequence and do not require additional validation for the Omicron (B.1.1.529) variant. Therefore, laboratories and test methods previously verified/validated for the testing of BNT162b2 original drug substance are considered suitable for testing of Omicron (B.1.1.529) variant.

2.3.S.4.4. Batch Analyses

Omicron (B.1.1.529) variant drug substance batches used for clinical trials, small scale development, large scale confirmatory, and stability are summarized in [Section 3.2.S.4.4 Batch Analyses \[Omicron \(B.1.1.529\) Variant\]](#).

A full drug substance genealogy can be found in [Section 3.2.S.2.6 Developmental History and Comparability \[Omicron \(B.1.1.529\) Variant\]](#).

2.3.S.4.5. Justification of Specification

The specification for Omicron (B.1.1.529) variant drug substance is based on an understanding of the control strategy and CQAs for the BNT162b2 original drug substance. There are no changes to the specifications for Omicron (B.1.1.529) variant drug substance when compared to BNT162b2 original drug substance. The attributes tested and associated acceptance criteria ensure the consistency of drug substance and linkage to clinical experience. This specification was established to ensure the quality, purity, potency/biological activity and safety of the commercial drug substance at release and during storage Specification-Setting Strategy.

Because there are no significant trends that would impact the shelf life of the drug substance

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the acceptance criteria used for stability over shelf life are the same as the acceptance criteria used for batch release. There are no changes to the specifications for Omicron (B.1.1.529) variant drug substance when compared to BNT162b2 original drug substance. In summary, the acceptance criteria in the drug substance specification reflect the current understanding of criticality of quality attributes, their impact on product performance, their stability, and the quality of the product used in clinical trials.