3.2.S.4.3. ENDOTOXIN

The validation of the bacterial endotoxins test, using the kinetic turbidimetric limulus amebocyte lysate (LAL) analytical procedure [redacted], the kinetic chromogenic LAL analytical procedures [redacted], 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:

- The criteria for the standard curve must be valid.
- The sample solution must not interfere with the test (e.g. inhibition/enhancement)
- 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:

- MVD = (endotoxin limit)/ (λ)
  Where,
  λ = [redacted] (label sensitivity of the lysate)

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

The validation results for the kinetic turbidimetric LAL method from [redacted] (Table 3.2.S.4.3-1 and Table 3.2.S.4.3-2), [redacted] (Table 3.2.S.4.3-3), and [redacted] (Table 3.2.S.4.3-4 and Table 3.2.S.4.3-5) are provided.

Table 3.2.S.4.3-1. Summary of Inhibition/Enhancement Data for BNT162b2 Drug Substance ([redacted])

Process Step	Endotoxin Limit	λ (EU/mL)	Calculated MVD	Qualified Dilution
Drug substance	≤12.5 EU/mL	[redacted]		

Table 3.2.S.4.3-2. Inhibition/Enhancement Results for BNT162b2 Drug Substance 4.2 1st ind

Batch Number	Sample Dilution	Spike Recovery (%)	Results (EU/mL)
20Y513C101	4.2 1st ind		
20Y513C201			
20Y513C301			

4.2 1st ind

Table 3.2.S.4.3-3. Inhibition/Enhancement Results for BNT162b2 Drug Substance 4.2 1st ind

Batch Number	Sample Dilution	Spike Recovery (%)	Results (EU/mL)
20E162001 4.2 1st ind 1071539)	4.2 1st ind		
20E162002 4.2 1st ind 1071542)			
20E162003 4.2 1st ind 1071544)			

4.2 1st ind

Table 3.2.S.4.3-4. Summary of Inhibition/Enhancement Data for BNT162b2 Drug Substance, 4.2 1st ind

Process Step	Endotoxin Limit	λ (EU/mL)	Calculated MVD	Validated Dilution
Drug substance	≤12.5 EU/mL	4.2 1st ind		

Table 3.2.S.4.3-5. Inhibition/Enhancement Results for BNT162b2 Drug Substance, 4.2 1st ind

Batch	Dilution	Results (EU/mL)	Spike Recovery (%)
Acceptance Criteria	**NA**	**NA**	**50-200%**
EP3344	Neat[a]	4.2 1st ind	NA
	4.2 1st ind		4.2 1st ind
EP3345	Neat[a]		NA
	4.2 1st ind		4.2 1st ind
EP3346	Neat		NA
	4.2 1st ind		4.2 1st ind

a. The neat sample did not meet acceptance criteria for recovery and is not appropriate for routine analysis.
Abbreviations: NA = not applicable

The validated dilution was determined 4.2 1st ind and is within the calculated MVD. The kinetic turbidimetric LAL analytical procedure is validated for its intended use at 4.2 1st ind

The qualification results for the kinetic chromogenic LAL method are provided from 4.2 1st ind (Table 3.2.S.4.3-6, Table 3.2.S.4.3-7 and Table 3.2.S.4.3-8).

Table 3.2.S.4.3-6. Endotoxin Concentration Results for Three Dilutions of BNT162b2 Drug Substance (4.2 1st ind)

Batch Number	Sample Dilution	Mean Positive Product Control (%)	Mean Endotoxin concentration (EU/mL)
CZ13-P020.2-DS	4.2 1st ind		

Table 3.2.S.4.3-7. Endotoxin Results for Holding Time of BNT162b2 Drug Substance 4.2 1st ind

Initial Spike Concentration (EU/mL)	Holding Time (time point / h)	Mean Positive Product Control (%)	Mean Spike Control Concentration (EU/mL)	Accuracy* (%)
4.2 1st ind	4.2 1st ind			

4.2 1st ind

Table 3.2.S.4.3-8. Endotoxin Results for Holding Time of BNT162b2 Drug Substance 4.2 1st ind (4.2 1st ind

Initial Spike Concentration (EU/mL)	Holding Time (time point / h)	Mean Positive Product Control (%)	Mean Spike Control Concentration (EU/mL)	Accuracy* (%)
4.2	4.2 1st ind			

4.2 1st ind

The validated dilution was determined 4.2 1st ind and is within the calculated MVD. The kinetic-chromogenic LAL analytical procedure is validated for its intended use at 4.2 1st ind

The validation results for the kinetic chromogenic LAL method are provided from 4.2 1st ind Scale (Table 3.2.S.4.3-9 and Table 3.2.S.4.3-10).

Table 3.2.S.4.3-9. Summary of Inhibition/Enhancement Data for BNT162b2 Drug Substance, 4.2 1st ind **,**

Process Step	Endotoxin Limit	λ (EU/mL)	Calculated MVD	Validated Dilution
Drug substance	≤12.5 EU/mL	4.2 1st ind		

Table 3.2.S.4.3-10. Inhibition/Enhancement Results for BNT162b2 Drug Substance, 4.2 1st ind [redacted],

Batch	Dilution	Results (EU/mL)	Spike Recovery (%)
Acceptance Criteria	**NA**	**NA**	**50-200%**
FH7784	Neat[a]	4.2 1st ind [redacted]	NA
	4.2 1st ind [redacted]		4.2 1st ind [redacted]
FJ1355	Neat[a]		NA
	4.2 1st ind [redacted]		NA[b]
			4.2 1st ind [redacted]
FJ5358	Neat[a]		NA
	4.2 1st ind [redacted]		4.2 1st ind [redacted]

[a] The neat sample did not meet acceptance criteria for recovery and is not appropriate for routine analysis.
[b] Sample replicate is invalid due to the spike recovery result falling outside of the acceptable range if 50-200%.
Abbreviations: NA = not applicable

The validated dilution was determined 4.2 1st ind [redacted] and is within the calculated MVD. The kinetic turbidimetric LAL analytical procedure is validated for its intended use at 4.2 1st ind [redacted] at 4.2 1st ind [redacted] Scale.

The qualification results for the kinetic chromogenic LAL method are provided from 4.2 1st ind [redacted] (Table 3.2.S.4.3-11 and Table 3.2.S.4.3-12)

Table 3.2.S.4.3-11. Summary of Inhibition/Enhancement Data for BNT162b2 Drug Substance 4.2 1st ind [redacted]

Process Step	Endotoxin Limit	λ (EU/mL)	Calculated MVD	Validated Dilution[a]
Drug Substance	≤ 12.5 EU/mL	4.2 1st [redacted]	[redacted]	[redacted]

Table 3.2.S.4.3-12. Inhibition/Enhancement Results for BNT162b2 Drug Substance
4.2 1st ind

Batch Acceptance Criteria	Dilution NA	Results (EU/mL) NA	Spike Recovery (%) 50-200%
FP5960	4.2 1st ind		
FP5961			
FT0774			

Abbreviations: NA = not applicable

The validated dilution was determined 4.2 1st ind and is within the calculated MVD. Additional qualified dilutions are 4.2 1st ind. The kinetic-chromogenic LAL analytical procedure is validated for its intended use at 4.2 1st ind