

### 3.2.S.4.3. BIOBURDEN

The validation of the bioburden analytical procedure was performed following the principles described in USP <61>, Ph. Eur. 2.6.12, and JP 4.05 for BNT162b2 drug substance (DS). The method validation (challenge recovery test) challenges the test method to ensure that the test articles are non-inhibitory to the recovery of inoculated microorganisms.

The samples tested for the presence of bioburden are generally expected to be non-bacteriostatic/non-fungistatic, or to be readily neutralized by membrane filtration. To demonstrate this, the bioburden assay was performed with the addition of a microbial inoculum composed of specified organisms (USP recommended). The challenge recovery testing was performed for each test article with each microorganism along with the appropriate inoculum control.

The acceptance criteria for this validation were as follows:

- 4.2 1st ind : Percent recovery of each microorganism for each test article must be between 4.2 1st ind
- 4.2 1st ind : In the presence of the test article, the mean count of each test organism may not differ by a factor greater than 2 from the value of the inoculum control.
- 4.2 1st ind The method is suitable if the counts from the sample differ by not more than a factor 2 from the control values, i.e. recovery rate of at least 4.2 1st ind must be given.
- 4.2 1st ind : The number of colonies on the duplicate test plate counts must be within 4.2 1st ind recovery of the results obtained for the number of colonies of the duplicate positive controls.
- 4.2 1st ind : The average microbial growth comparisons between the test groups and the positive control groups must be 4.2 1st ind

Three batches of BNT162b2 DS (target concentration of 4.2 1st ind), 10 mL in at least duplicates per organism, were tested for the validation of the method.

The validation results 4.2 1st ind are provided in Table 3.2.S.4.3-1, Table 3.2.S.4.3-2 Table 3.2.S.4.3-3, Table 3.2.S.4.3-4, Table 3.2.S.4.3-5 and Table 3.2.S.4.3-6 respectively.

**Table 3.2.S.4.3-1. BNT162b2 Challenge Recovery Testing Results for Drug Substance** 4.2 1st ind

| Batch <sup>b</sup> | Organism <sup>b</sup>  | Recovery (%) |
|--------------------|------------------------|--------------|
| 20Y513C101         | <i>A. brasiliensis</i> | 4.2 1st ind  |
|                    | <i>B. subtilis</i>     |              |
|                    | <i>C. albicans</i>     |              |
|                    | <i>P. aeruginosa</i>   |              |
|                    | <i>S. aureus</i>       |              |
| 20Y513C201         | <i>A. brasiliensis</i> |              |
|                    | <i>B. subtilis</i>     |              |
|                    | <i>C. albicans</i>     |              |
|                    | <i>P. aeruginosa</i>   |              |
|                    | <i>S. aureus</i>       |              |
| 20Y513C301         | <i>A. brasiliensis</i> |              |
|                    | <i>B. subtilis</i>     |              |
|                    | <i>C. albicans</i>     |              |
|                    | <i>P. aeruginosa</i>   |              |
|                    | <i>S. aureus</i>       |              |

4.2 1st ind

b. Challenge recovery testing was performed for each test article with each microorganism, as well as corresponding inoculum control in duplicate.

**Table 3.2.S.4.3-2. BNT162b2 Challenge Recovery Testing Results for Drug Substance** 4.2 1st ind

| Batch                              | Organism               | Recovery factor |
|------------------------------------|------------------------|-----------------|
| 20E162001<br>(4.2 1st ind 1071539) | <i>A. brasiliensis</i> | 4.2 1st ind     |
|                                    | <i>B. subtilis</i>     |                 |
|                                    | <i>C. albicans</i>     |                 |
|                                    | <i>P. aeruginosa</i>   |                 |
|                                    | <i>S. aureus</i>       |                 |
| 20E162002<br>(4.2 1st ind 1071542) | <i>A. brasiliensis</i> |                 |
|                                    | <i>B. subtilis</i>     |                 |
|                                    | <i>C. albicans</i>     |                 |
|                                    | <i>P. aeruginosa</i>   |                 |
|                                    | <i>S. aureus</i>       |                 |
| 20E162003<br>(4.2 1st ind 1071544) | <i>A. brasiliensis</i> |                 |
|                                    | <i>B. subtilis</i>     |                 |
|                                    | <i>C. albicans</i>     |                 |
|                                    | <i>P. aeruginosa</i>   |                 |
|                                    | <i>S. aureus</i>       |                 |

4.2 1st ind

b. Challenge recovery testing was performed for each test article with each microorganism, as well as corresponding inoculum control in triplicate.

**Table 3.2.S.4.3-3. BNT162b2 Challenge Recovery Testing Results for Drug Substance** 4.2 1st ind

| Batch           | Organism                      | Recovery (%) |
|-----------------|-------------------------------|--------------|
| CZ12-P0202.2-DS | <i>B. subtilis</i>            | 4.2 1st ind  |
|                 | <i>S. aureus</i>              |              |
|                 | <i>P. aeruginosa</i>          |              |
|                 | <i>C. albicans</i> (Caso)     |              |
|                 | <i>C. albicans</i> (Sab.)     |              |
|                 | <i>A. brasiliensis</i> (Caso) |              |
|                 | <i>A. brasiliensis</i> (Sab.) |              |

**Table 3.2.S.4.3-4. BNT162b2 Challenge Recovery Testing Results** 4.2 1st ind, 4.2 1st ind

| Organism               | Trial <sup>a</sup> | Results (CFU Average/Plate) |             | Recovery (%) |
|------------------------|--------------------|-----------------------------|-------------|--------------|
|                        |                    | Positive Control            | Test Sample |              |
| <i>B. subtilis</i>     | 1                  | 4.2 1st ind                 |             |              |
|                        | 2                  |                             |             |              |
|                        | 3                  |                             |             |              |
| 4.2 1st ind            | 1                  |                             |             |              |
|                        | 2                  |                             |             |              |
|                        | 3                  |                             |             |              |
| <i>P. aeruginosa</i>   | 1                  |                             |             |              |
|                        | 2                  |                             |             |              |
|                        | 3                  |                             |             |              |
| 4.2 1st ind            | 1                  |                             |             |              |
|                        | 2                  |                             |             |              |
|                        | 3                  |                             |             |              |
| <i>S. aureus</i>       | 1                  |                             |             |              |
|                        | 2                  |                             |             |              |
|                        | 3                  |                             |             |              |
| 4.2 1st                | 1                  |                             |             |              |
|                        | 2                  |                             |             |              |
|                        | 3                  |                             |             |              |
| <i>C. albicans</i>     | 1                  |                             |             |              |
|                        | 2                  |                             |             |              |
|                        | 3                  |                             |             |              |
| 4.2 1st ind            | 1                  |                             |             |              |
|                        | 2                  |                             |             |              |
|                        | 3                  |                             |             |              |
| <i>A. brasiliensis</i> | 1                  |                             |             |              |
|                        | 2                  |                             |             |              |
|                        | 3                  |                             |             |              |
| 4.2 1st                | 1                  |                             |             |              |
|                        | 2                  |                             |             |              |
|                        | 3                  |                             |             |              |

a. Batches EP3334, EP3335 and EP3336 were tested in trials 1, 2 and 3, respectively.

4.2 1st ind

**Table 3.2.S.4.3-5. BNT162b2 Challenge Recovery Testing Results** (4.2 1st ind  
[REDACTED])

| Organism               | Trial | Results (CFU Average/Plate) |             | Recovery (%) |
|------------------------|-------|-----------------------------|-------------|--------------|
|                        |       | Positive Control            | Test Sample |              |
| <i>B. subtilis</i>     | 1     | 4.2 1st ind [REDACTED]      |             |              |
| <i>B. cereus</i>       | 1     |                             |             |              |
| <i>P. aeruginosa</i>   | 1     |                             |             |              |
| 4.2 1st ind [REDACTED] | 1     |                             |             |              |
| <i>S. aureus</i>       | 1     |                             |             |              |
| 4.2 1st ind [REDACTED] | 1     |                             |             |              |
| <i>C. albicans</i>     | 1     |                             |             |              |
| 4.2 1st ind [REDACTED] | 1     |                             |             |              |
| <i>A. brasiliensis</i> | 1     |                             |             |              |
| 4.2 1st ind [REDACTED] | 1     |                             |             |              |

**Table 3.2.S.4.3-6. BNT162b2 Challenge Recovery Testing Results**

| Organism                                        | Batch                | Results (CFU Average/Plate)     |                       | Recovery (%) |
|-------------------------------------------------|----------------------|---------------------------------|-----------------------|--------------|
|                                                 |                      | Positive Control                | Test Sample           |              |
| Media: 4.2 1st ind                              | Soy Agar 4.2 1st ind | Incubation: 30-35 °C for 5 days |                       |              |
| <i>S. aureus</i>                                | FP5960               | 4.2 1st ind                     |                       |              |
|                                                 | FP5961               |                                 |                       |              |
|                                                 | FT0774               |                                 |                       |              |
| <i>B. subtilis</i>                              | FP5960               |                                 |                       |              |
|                                                 | FP5961               |                                 |                       |              |
|                                                 | FT0774               |                                 |                       |              |
| <i>P. aeruginosa</i>                            | FP5960               |                                 |                       |              |
|                                                 | FP5961               |                                 |                       |              |
|                                                 | FT0774               |                                 |                       |              |
| 4.2 1st ind                                     | FP5960               |                                 |                       |              |
|                                                 | FP5961               |                                 |                       |              |
|                                                 | FT0774               |                                 |                       |              |
| 4.2 1st ind                                     | FP5960               |                                 |                       |              |
|                                                 | FP5961               |                                 |                       |              |
|                                                 | FT0774               |                                 |                       |              |
| <i>C. albicans</i>                              | FP5960               |                                 |                       |              |
|                                                 | FP5961               |                                 |                       |              |
|                                                 | FT0774               |                                 |                       |              |
| <i>A. brasiliensis</i>                          | FP5960               |                                 |                       |              |
|                                                 | FP5961               |                                 |                       |              |
|                                                 | FT0774               |                                 |                       |              |
| Media / Incubation: Sabouraud Dextrose Soy Agar |                      | 4.2 1st ind                     | / 20-25 °C for 5 days |              |
| <i>C. albicans</i>                              | FP5960               | 4.2 1st ind                     |                       |              |
|                                                 | FP5961               |                                 |                       |              |
|                                                 | FT0774               |                                 |                       |              |
| <i>A. brasiliensis</i>                          | FP5960               |                                 |                       |              |
|                                                 | FP5961               |                                 |                       |              |
|                                                 | FT0774               |                                 |                       |              |

The results of the validation study (challenge recovery) demonstrate the bioburden method is effective in recovering microorganisms from the test article. The analytical procedure is validated for its intended use.