3.2.P.3.5. VERIFICATION OF IN-PROCESS TEST METHODS [PUURS]

Descriptions of the validation of the analytical procedures used for in-process control (IPT-C) are provided below.

3.2.P.3.5.1. pH

The pH is measured potentiometrically during preparation of the buffer using calibrated pH meters. This method meets compendial requirements.

3.2.P.3.5.2. RNA Content by Fluorescence Assay

A summary of the method validation for in-process testing is detailed in Section 3.2.P.5.3 Fluorescence Assay.

3.2.P.3.5.3. Bioburden

The bioburden analytical procedure (performed following the principles described in USP <61>, Ph. Eur. 2.6.12, and JP 4.05, using membrane filtration) was verified for BNT162b2 drug product (DP) in-process samples. The method verification (challenge recovery test) challenges the test method to ensure that the test articles are non-inhibitory to the recovery of inoculated microorganisms. Verification of the pre-sterile filtration sample is detailed in this section.

The test article tested for the presence of bioburden is 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 (target titer of not more than 100 colony forming units (CFUs)) composed of compendial -recommended microorganisms and in-house microorganisms. Verification of the bioburden method consisted of challenge-recovery testing on 3 pre-sterile filtration test articles. The method verification consists of:

- Neutralizer efficacy; which is assessed by adding a low level of inoculum of a challenge organism to the test article and demonstrates that recovery is not inhibited by product residues on the filter.
- Neutralizer toxicity; which is assessed using a neutralizer control (without product) and demonstrates that recovery is not inhibited by the use of neutralization agent(s) and/or filter type.
- Negative controls (blanks); demonstrate that the materials used (e.g. media, filter, diluent) are free from contamination.
- Inoculum controls; demonstrate the micro-organisms being used are suitable for use.

Verification of the bioburden method consisted of challenge-recovery testing on 3 different test articles (material from bulk drug product lots EG5447, EH9979 and EK2538).

For the challenge recovery testing to be verified, the following acceptance criteria must be met:

- The inoculum control count of all test organisms on Tryptic Soy Agar (TSA) and Sabouraud Dextrose Agar + Chloramphenicol (SDAC) has to be between [redacted] CFU.
- The blank of the neutralizer control may not exhibit growth on TSA and SDAC.
- The blank of the product test on TSA and SDAC has to be [redacted] of the lowest inoculum control count of all test organisms on TSA and SDAC.
- Neutralizer efficacy microbial recovery from the inoculated product count has to be at least [redacted] compared to the inoculum control.
- Neutralizer toxicity microbial recovery from the neutralizer control count has to be at least [redacted] compared to the inoculum control.

3.2.P.3.5.3.1. Bioburden Verification Data

For each verification run, 100 mL of the pre-sterile filtration sample was inoculated with not more than 100 CFUs of the challenge microorganism and filtered. This procedure was repeated for each challenge organism listed below:

- Bacterial test organisms: *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Bacilus subtilis* ATCC 6633, *Ralstonia picketti* (UP 71) in-house isolate common in water samples, *Stenotrophomonas maltophilia* (UP 07) in house isolate common in bioburden samples.
- Fungal test organisms: *Candida albicans* ATCC 10231, *Aspergillus brasiliensis* ATCC 16404.

The summary data that supports the verification of the pre-sterile filtration material is presented in the tables below.

Table 3.2.P.3.5-1. Summary Data for Pre-sterile Filtration Bioburden Challenge Recovery: Trial 1 Using Lot EG5447

Microorganism	Inoculum Control (CFU) (A)	Neutralizer Control Count (CFU) (B)	Inoculated Product Control Count (CFU) (C)	Neutralizer Toxicity (%) (B/A) × 100%	Neutralizer Efficacy (%) (C/A) × 100%	Acceptance Criteria
Total Aerobic Microbial Count: Tryptic Soy Agar, 30-35 °C, ≤3 days						
Blank	NA	0	0	NA	NA	B = 0 CFU C [redacted] of lowest inoculated product control count
S. aureus	[redacted]					A between [redacted] CFU (B/A) × 100% = [redacted] % (C/A) × 100% = [redacted] %
R. pickettii						
S. maltophilia						
B.subtilis						
P. aeruginosa						
C. albicans						
A. brasiliensis						
Total Yeast and Mold Count: Sabouraud Dextrose Agar + Chloramphenicol, 20 -25 °C, ≤5 days						
Blank	NA	0	0	NA	NA	B = 0 CFU C [redacted] of lowest inoculated product control count
C. albicans	[redacted]					A between [redacted] CFU (B/A) × 100% = [redacted] % (C/A) × 100% = [redacted] %
A. brasiliensis						

NA = not applicable

Table 3.2.P.3.5-2. Summary Data for Pre-sterile Filtration Bioburden Challenge Recovery: Trial 2 Using Lot EH9979

Microorganism	Inoculum Control (CFU) (A)	Neutralizer Control Count (CFU) (B)	Inoculated Product Control Count (CFU) (C)	Neutralizer Toxicity (%) (B/A) × 100%	Neutralizer Efficacy (%) (C/A) × 100%	Acceptance Criteria
Total Aerobic Microbial Count: Tryptic Soy Agar, 30-35 °C, ≤3 days						
Blank	NA	0	0	NA	NA	B = 0 CFU C [redacted] of lowest inoculated product control count
S. aureus	[redacted]					A between [redacted] CFU (B/A) × 100% = [redacted]% (C/A) × 100% = [redacted]%
R. pickettii						
S. maltophilia						
B.subtilis						
P. aeruginosa						
C. albicans						
A. brasiliensis						
Total Yeast and Mold Count: Sabouraud Dextrose Agar + Chloramphenicol, 20 -25 °C, ≤5 days						
Blank	NA	0	0	NA	NA	B = 0 CFU C [redacted] of lowest inoculated product co count
C. albicans	[redacted]					A between [redacted] CFU (B/A) × 100% = [redacted]% (C/A) × 100% = [redacted]%
A. brasiliensis						

NA = not applicable

Table 3.2.P.3.5-3. Summary Data for Pre-sterile Filtration Bioburden Challenge Recovery: Trial 3 Using Lot EK2538

Microorganism	Inoculum Control (CFU) (A)	Neutralizer Control Count (CFU) (B)	Inoculated Product Control Count (CFU) (C)	Neutralizer Toxicity (%) (B/A) × 100%	Neutralizer Efficacy (%) (C/A) × 100%	Acceptance Criteria
Total Aerobic Microbial Count: Tryptic Soy Agar, 30-35 °C, ≤3 days						
Blank	NA	0	0	NA	NA	B = 0 CFU C [redacted] of lowest inoculated product control count
S. aureus	[redacted]					A between [redacted] CFU (B/A) × 100% = [redacted] % (C/A) × 100% = [redacted] %
R. pickettii						
S. maltophilia						
B.subtilis						
P. aeruginosa						
C. albicans						
A. brasiliensis						
Total Yeast and Mold Count: Sabouraud Dextrose Agar + Chloramphenicol, 20 -25 °C, ≤5 days						
Blank	NA	0	0	NA	NA	B = [redacted] CFU C [redacted] of lowest inoculated product control count
C. albicans	[redacted]					A between [redacted] CFU (B/A) × 100% = [redacted] % (C/A) × 100% = [redacted] %
A. brasiliensis						

NA = not applicable

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