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Application Note

Sterikon® plus Bioindicator

Incubation Time Validation

Information about the effectiveness of sterilizers can be provided by bioindicators (BIs). They are used to monitor the adequacy of the sterilization process. BIs contain a known number of microorganism that have a known resistance to the mode of sterilization. A subsequent growth or a failure in growth of the specific microorganism indicates the adequacy of sterilization.

The Sterikon® plus Bioindicators are self-contained and consist of a glass ampoule filled with a nutrient broth, sugar, a pH indicator and spores of a non-pathogenic organism, *Geobacillus stearothermophilus* ATCC 7953 (sporulation optimized). Its thermal resistance is such that the spores are completely killed after 15 minutes when heated in compressed steam at a temperature of 121 °C (± 0.5). At lower temperatures or lower exposure time a small number of spores can survive.

Sterikon® plus Bioindicators are fully compliant with the requirements of both European and United States Pharmacopoeia. They are not registered as medical devices (IvD), and full compliance with ISO 11138 and FDA Guidance for Industry about Biological Indicators is not part of the product claim. Nevertheless, we strive to fulfill as many of the requirements listed in the above mentioned guidelines as possible and applicable.

The recommended incubation time for Sterikon® plus Bioindicator is 48 hours 60 °C ± 2 °C. As the FDA generally recommends seven days as the conventional incubation time for BIs used to monitor traditional sterilization processes a study was conducted to validate the shorter incubation time for BIs after the sterilization process following the methodology specified in the “Guidance for Industry and FDA Staff — Biological Indicator (BI) Premarket Notification [510(k)] Submission” (October 4, 2007).

The validation study (performed by Quality Control Darmstadt, Germany) described in this Application Note was conducted to confirm the incubation time of 48 hours to be sufficient according to appropriate methodology.

Material and Equipment

Table 1: Material

Cat. No.	Product Name	Batches Tested
1.10274	Sterikon® plus Bioindicator	3

Method

Three batches of Sterikon® plus Bioindicator were prepared according to the manual. To simulate a failed sterilization process, 18 biological indicators (BIs) of each batch were placed in an oil bath at 121°C (± 0.5) for 13 minutes. This procedure was repeated 6 times per batch, to obtain an overall number of 108 BIs tested per batch.

Afterwards the BIs were incubated at 60 °C ± 2 °C for seven days. The number of positive indicators (growth of spores) was recorded daily. A positive test is indicated by a color change from violet to yellow.

Results

According to the recommended methodology from the FDA, a partial sterilization cycle in which 30–80% of the BIs survive, needed to be identified. A partial cycle is characterized by defined sterilization parameters, where just the sterilization time is changed.

For batch #1 and #3 a percentage of 31.5 was achieved, for batch #2, 34.3% of the BIs were positive. Thus the recommended value range was met by all three batches, and they can be considered as valid cycles.

After 1 day a growth percentage of 100% (34 of 34 BIs were positive) was obtained for batch #1 and batch #3. For batch #2 after 1 day a growth percentage of 97.4% (37 of 38 BIs were positive) and after 2 days of 100% (38 of 38 BIs positive) was obtained (see **Table 2**).

Table 2: Validation Test Data of 3 Sterikon® plus Bioindicator Batches

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Batch #1							
Numerator	34	34	34	34	34	34	34
Denominator	34	34	34	34	34	34	34
Percent Growth	100%	100%	100%	100%	100%	100%	100%
Batch #2							
Numerator	37	38	38	38	38	38	38
Denominator	38	38	38	38	38	38	38
Percent Growth	97.4%	100%	100%	100%	100%	100%	100%
Batch #3							
Numerator	34	34	34	34	34	34	34
Denominator	34	34	34	34	34	34	34
Percent Growth	100%	100%	100%	100%	100%	100%	100%

Interpretation

The reduced incubation time of 2 days (48 hours) is valid as all partial cycles achieved 97-100% growth after 2 days, and FDA requirements are met.

All data suggests that users can safely rely on this conveniently short incubation time to support their product release without unnecessary delay.

Further Reading and Information

European Directorate for the Quality of Medicines and Healthcare. (2014): The European Pharmacopoeia. 8th Ed. Chapter 5.1.2 Biological Indicators of Sterilization

FDA Guidance for Industry and FDA Staff (2007): Biological Indicator (BI) Premarket Notification [510(k)] Submissions

PDA Technical Report No. 51 (2010): Biological Indicators for Gas and Vapor-Phase Decontamination Processes: Specification, Manufacture, Control and Use

United States Pharmacopoeia 29 (2006): <1035> Biological Indicators for Sterilization

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