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Reduced Incubation Times for Biological Indicators

by Robert Bradley and Garrett Krushefski

You just received your latest order of biological indicators (BIs). You ordered two different items, a bag of *Geobacillus stearothermophilus* cotton threads and a box of ProTest Steam biological indicators. Upon reviewing the labeling and instructions for use you find that both indicators are for the same sterilization process, contain the same species and quantity of bacteria but have vastly different incubation periods. The threads must be incubated for 7 days and the ProTest Steam is incubated for 24 hours. Why the difference?

Many BI manufacturers have established reduced incubation times (RITs) for some of the biological indicators that they offer. In most instances, manufacturers will establish RITs for products used in healthcare facilities as well as high volume products used in the manufacturing industry. Some examples of these products are:

- ProTest Steam: 24-hour RIT
- EZTest GAS: 48-hour RIT
- ProSpore Ampoule: 48-hour RIT
- SporeStrip-Steam[1]: 24-hour RIT

These RITs are developed using a guidance set forth by the FDA's Center for Device and Radiological Health (CDRH). The document 'Guidance for Industry and FDA Staff - Biological Indicator (BI) Premarket Notification 510(k) Submissions' establishes a test protocol for qualifying reduced incubation times. While the CDRH guidance is directed toward biological indicators used in Healthcare facilities historically it has generally been adopted by the manufacturing industry as well.

The guidance requires BI manufacturers to expose a total of 300 BIs (100 BIs from three different lots) to partial conditions in the appropriate sterilization process that produce a mixture of BIs that are positive and negative for growth. The guidance requires that the overall number of positive BIs from each lot be within the range of 30-80% of the total number of BIs. Those BIs are graded daily and at the end of the seven (7) day incubation period. The daily results are compared against the final result at seven (7) days.

Using the number of BIs that test positive on day 7 as the base of 100% grow out, the greatest number of days of incubation time to obtain more than 97% positive BIs (based on the 7-day incubation time) in any one of the three lots is the minimum incubation time for the BI. The table below will help illustrate this concept.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
BI Lot #1							
Numerator	46	76	77	78	78	78	78
Denominator	78	78	78	78	78	78	78
Percent Growth	58.97%	97.44%	98.72%	100%	100%	100%	100%
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
BI Lot #2							
Numerator	52	65	65	65	65	65	65
Denominator	65	65	65	65	65	65	65
Percent Growth	80.00%	100%	100%	100%	100%	100%	100%
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
BI Lot #3							
Numerator	48	48	48	49	49	49	49
Denominator	49	49	49	49	49	49	49
Percent Growth	97.96%	97.96%	97.96%	100%	100%	100%	100%

Table 1 – Reduced Incubation Time Qualification Testing

The numerator represents the total number of positive BIs each day. The denominator represents the total number of BIs positive for growth after seven (7) days. In this example, a 48-hour RIT has been qualified regardless of the 24-hour result obtained for BI Lot #3.

As stated earlier, 'The CDRH guidance is directed toward biological indicators used in Healthcare facilities and historically it has generally been adopted by the manufacturing industry as well'. It has recently come to the attention of Mesa Laboratories that the FDA CDER (Center for Drug Evaluation and Research) might not necessarily endorse or condone the test protocol published by the FDA CDRH; citing that the test parameters set forth in the CDRH protocol are applicable for BIs used to monitor sterilization processes in healthcare facilities, but are not appropriate for BIs that are used in a manufacturing setting.[2] A recent FDA CDER communication to an end user that was eventually forwarded to Mesa indicated that:

'CDER does not generally recognize the acceptance criterion that the minimum incubation time is the greatest number of days to obtain more than 97% positive BIs subjected to a partial cycle…'.

If you are in the pharmaceutical or manufacturing industries what does this mean for you? The CDER doesn't recognize the CDRH guidance but they don't have a guidance of their own. CDER is indicating that manufacturers of sterile drug products should adhere to the 7-day recommendation that appears in 11138-1. '*ISO 11138-1: Sterilization of healthcare products – Biological indicators-Part 1: General Requirements*' states the following in sections 7.3.1 and 7.3.2:

- The incubation time and temperature shall be validated.
- BI manufacturer shall provide instructions for incubation.
- Incubation period is commonly recognized to be 7 days for established sterilization processes (such as moist heat and ethylene oxide).
- For novel sterilization methods, a 14-day incubation period shall be used as the reference incubation period on which to base the validation.
- NOTE: National or regional requirements for incubation period validation may also exist.

'*ISO 14161: Sterilization of healthcare products – biological indicators-Guidance for selection, use and interpretation of results*' section 12.3.3 acknowledges that a BI manufacturer may validate a reduced incubation time that does not have to be repeated by the end user as long as the end user uses the BI in the same sterilizing agent for which the BI was designed and validated. Considering the verbiage that appears in section 12.3.3 of 14161, it is understandable that BI users that are not in a healthcare setting would follow the reduced incubation time label instructions and would have no indication that doing so would subject themselves to regulatory scrutiny.

However, the recent revelations about the stance of CDER on the concept of RIT suggest that BI users in the pharmaceutical/manufacturing industry would be prudent to disregard that which appears in ANSI/AAMI/ISO 14161, section 12.3.3 and incubate their exposed BIs for a full seven days.

Alternatively, if you find yourself in a situation where your regulatory body fails to recognize the established RIT for a biological indicator or possibly in preparation for that day to come, one might choose to incubate and score the BIs at the reduced incubation time that appears in the instructions and keep the BIs incubating for the remainder of the 7-day period at which time the BIs are again scored for results. Reading the exposed BIs twice (once at the RIT and again at the conclusion of day 7) would allow the user to make appropriate product release decisions without undue delay and the availability of day 7 data would satisfy regulatory bodies.

[1] When used in conjunction with OMF's modified Tryptic Soy Broth/BCP.
[2] Mesa has not had direct communication with the CDER on this topic. All correspondence has occurred through third parties that have relayed the information to Mesa.

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