Spore News

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Why Biological Indicators Survive a Validated Cycle

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When biological indicators (BIs) are exposed in a validated sterilization cycle, it is expected that all test units will be sterile. A review of the cycle performance shows that all critical parameters were achieved. Regardless, the BIs are activated and incubated as usual and for the next several days of incubation the exposed BI results are recorded. Hopefully you will never see a positive BI (yellow color) except in the case of positive controls. But where do you turn if one day you remove the samples from the incubator and staring back at you is an unexpected positive (yellow) test unit?

Part of being a manufacturer of high quality biological indicators (BI) is providing excellent support and service to the users of these products. Distributing a quality product and subsequent failure to answer questions regarding its proper use and interpretation of results falls short of what it means to be a quality manufacturer.

Recently I heard from a client who expressed the following concern about EZTest® Steam, self-contained biological indicators. The sterilization cycle in question was 7-minutes at 134°C. The exposed indicators tested negative (purple) at 48-hours of incubation. A few of the exposed units would, however, test positive (yellow) at subsequent post 48-hour observation. Finally, the client mentioned that in the past they used EZTest Steam with population of 10^5 per unit without experiencing "failure." They have since switched to EZTest 10^6 and occasionally see the post 48-hour positive unit appear.

To have a 48-hour incubation time claim (or any incubation time less than seven days) on a BI, the manufacturer must validate the claim by performing the FDA protocol for reduced incubation time (RIT) test.[1] This test, which must be performed in triplicate using a minimum of three separate lots, requires that one hundred BIs be exposed to a partial sterilization cycle such that 30 to 80 of the 100 exposed indicators test positive. The exposed BIs are incubated with the number of positive units being recorded at regular intervals throughout the seven days. The 48-hour incubation claim is valid if the number of positive units recorded at 48-hours divided by the number of positive units recorded at seven days is greater than 0.97. As an example, if 78 positives are observed

[1] RIT testing requirements can be found in The Center for Devices and Radiological Health, FDA Guide for Validation of Biological Indicator Incubation Time, document control number 98984.

on day seven, than at least 76 positives need to be recorded by 48-hours. 76 ÷ 78 = 0.974. If 75 or fewer units tested positive at 48-hours, the product has failed to perform according to the parameters of the RIT test such that 75 ÷ 78 = 0.962.

Knowing the specifics of the RIT test, one can see that it is possible for additional positive units to appear should incubation be allowed to continue beyond the label claim. However, the RIT test is designed to target the "quantal zone" where some of the exposed indicators test negative and some test positive. Routine sterilization cycles are not developed to target this zone. Validated and approved cycles are designed to deliver lethality well in excess of the quantal zone where complete inactivation of the spore population is rendered.

When one has achieved this quantal zone, the viable spore population of each individual indicator has been decreased to zero, one, two or very few surviving injured spores. Individual units that have zero surviving spores will test negative at 48-hours, seven (7) days and beyond. Individual indicators that have one or more surviving spores will test positive *usually* by 48-hours or less. The final consideration would be indicators that have one or two injured spores that may or may not be able to germinate, repair the damage caused by the sterilant, grow, reproduce, metabolize the nutrients in the media, thus producing acidic byproducts which causes the shift in the pH indicator and the yellow appearance. For these specific indicators, it is questionable if the unit will test positive or negative. Furthermore, the potential exists that due to the severity of the spore injury, proliferation and reproduction may be delayed and thus result in a negative unit at 48-hours that eventually turns yellow post 48-hours. This phenomenon will only occur in the quantal zone. Longer exposure duration to the sterilant surpasses the quantal zone and complete kill of all spores is achieved. As stated above, if zero spores survive, the unit will test negative at seven days and beyond.

A review of the data that were obtained in assessing the resistance performance for the lot in question showed evidence of the potential for additional post 48-hour positives. A total of 940 EZTest indicators were processed in a steam Biological Indicator Evaluator Resistometer (BIER) to assess the D-values at 121°, 124°, 129°, 132°, 134° and 135°C. Of the 940 units consumed, only six additional positives appeared following the 48-hour observation. All of the additional positives were from exposure durations in the quantal zone. For all exposures where complete inactivation was observed at 48-hours, zero positive units appeared throughout the seven day incubation.

While conducting D-value assessment at 134°C, complete kill was achieved in the 2.0, 2.5, 3.0, and 3.5-minute exposure. As you can see, under no circumstances should the client ever see a positive test unit **IF** the sterilizer was delivering the prescribed lethality to the biological indicator that corresponds to 7.0-minutes at 134°C saturated steam. The fact that the client was experiencing additional positives post 48-hours clearly indicates that the sterilization cycle was delivering a lethal dose of steam that was equivalent to what was delivered in the quantal zone. All of the client's exposed indicators were negative at 48-hours and then a few turned positive post 48-hours. This suggests that the

lethality was close to the end of the quantal zone and similar to our 1.5 or 2.0-minute BIER cycle.

The question still remains, why are BIs testing positive in a 134°C, 7-minute cycle? There are a few reasons why a biological indicator with calibrated performance could test positive when exposed to this cycle:

- The temperature probe indicates 134°C but is in error and a substantially lower temperature is actually prevailing within the sterilization chamber.
- The temperature probe indicates 134°C and is accurate but is not representative of the conditions that are prevailing in the immediate environment in which the BI is placed. Packaging, wrapping, and/or position within the load may be impeding steam penetration to or air removal from the location of the BI.
- The pressure probe is indicating a pressure that corresponds to saturated steam conditions at 134°C (i.e. 44.10 psia) but is in error and the actual pressure that is prevailing within the sterilization chamber is different.
- The pressure probe is accurate, but as a result of packaging, wrapping and or position within the load, the reading is not representative of the conditions that are prevailing in the immediate environment in which the BI is placed.
- Removal of air from the chamber (and thus the EZTest unit) and/or penetration of steam to the inoculated carrier within the EZTest unit is being impeded. This is the most common problem with high temperature, short cycle exposures and it is often exasperated due to the packaging/wrapping used for the cycle. If air removal and/or steam penetration is impeded in any way, the spores will respond to the prevailing conditions and integrate the lethality being delivered more precisely than any physical measurement. (Temperature and pressure probes are incapable of distinguishing between saturated steam and dry heat.) The self-contained EZTest indicator is an excellent BI to detect such cycle inefficiencies because it will function as an air trap. Unlike the permeable glassine packaging of the STRIP BI, the only location for air/steam exchange to occur in the EZTest is through the vented cap. As a result of the limited exchange interface, EZTest creates a "worst case scenario" condition and thus can detect air removal/steam penetration inefficiencies better than any other type of biological indicator.[2]
- Another possibility is that the exposed and incubated unit is being contaminated during the incubation. The contaminant is responsible for the change in media color and one has false-positives in a unit where complete spore inactivation was in fact achieved. Shaking, dropping or inverting the activated indicator at any time during the incubation could cause the filter paper in the cap to become wetted and thus the potential for introduction of a contaminant has been increased.

[2] Users should also be aware that the EZTest BI should be placed in a horizontal orientation during exposure. Such orientation serves to facilitate both the removal of air from the BI as well as subsequent steam penetration to the inoculated spore carrier. A vertical orientation (cap up) can lead to air retention in the BI and formation of an air pocket, especially in gravity displacement sterilization systems (those that do not employ a pre-vacuum to facilitate air removal).

The post-processing contamination is a scenario that must be considered. However, in this specific case, there was one statement that makes this scenario less probable. In the past, the user never had problems with the X10^5 spore population. It was only after the switch to the X10^6 EZTest that the problem surfaced. This information suggests that the cycle being used has been delivering a marginal lethality that was capable of inactivating the lower population BI. When the population was increased by a factor of ten, the marginal lethality became apparent in that one occasional survivor was obtained.

Essentially, the BI was performing exactly as it should. This is the purpose of a biological indicator and why companies producing sterile goods are required to use them; it serves to demonstrate that the conditions necessary to achieve sterilization were present within the chamber AND that the biological results agree with what the probes are indicating. Remember, the word "sterile" is defined as the absence of all viable microbial organisms; the condition is not defined based upon numerical temperature, pressure, and Fo values. Should you ever find yourself in the situation where the probes indicate conditions that would render killed BIs, yet the units are testing positive, remember that regardless of the expected lethality, the microorganism was in the sterilizing chamber and is now growing in the incubator. Obviously, the delivery of sterilant to the BI was insufficient.

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