Performance Comparison for Three Types of Biological Indicators Used in Steam Sterilization Processes: Spore Strips, Crushable Self-contained, and Sealed Glass Ampoule

Written By Dr. Robert Ramaley (Univ. NE Med CTR), Mia Ware, Dan Pearson, and Christopher R. Dwyer* (Raven Biological Laboratories)

*Direct questions to this Author

Phone # 800-728-5702 or 1-402-593-0781
Fax # 1-402-593-0995 or 1-402-593-0921
Email: ravenbio@aol.com

Regulatory officials and sterilization experts have voiced concerns regarding the appropriateness of using a Biological Indicator (BI) Ampoule interchangeably with spore strips or other approved self-contained Biological Indicators (BIs). They argued spores in a sealed glass ampoule do not have direct contact with the steam, and this lack of direct contact with the sterilant caused the Ampoule to behave differently than other types of BIs. There was no scientific data to support this argument, only the belief that since the spores do not have direct contact with the steam, the Ampoule should not be used in porous load cycles because a "poor quality steam environment" might not be detected by the Ampoule. This argument disregards the fact that the Ampoule BIs are tested for population, Dvalue and Zvalue by the same standardized methods and equipment that are used to test other BIs. The following report will describe various tests and data collected to determine if the Ampoule BI behaves equivalently to spore strips and other self-contained BIs.

Background: Biological Indicators (BIs) are used to determine whether a sterilizer has delivered a lethal cycle. Evaluation of resistant, spore-forming microorganisms processed through steam cycles gives the operator a direct measurement of the lethality delivered by the sterilizer during that particular cycle. The organisms used are of known quantity (population) and resistance (Dvalue). The organisms are packaged in such a way as to allow

the sterilant access to the spores, and allow for either enumeration or recovery of surviving organisms. Following are three packaging configurations for Bis used in steam cycles:

1) Spore Strip - spores are inoculated onto filter paper and then packaged in a glassine paper pouch or envelope. The glassine paper allows the penetration of sterilant, and it also keeps the strip from being contaminated after the sterilization cycle. After exposure in the sterilizer, the spore strip is transferred from the glassine into a test tube of recovery medium and incubated to check for sterility.
2) Self-contained with a media ampoule separate from the spores (e.g. Attest, Prospore2, Verify) - Spores are inoculated onto filter paper. The filter paper is placed into a plastic tube along with a sealed glass ampoule containing the recovery media. The tube is then capped, but the cap must allow direct penetration of sterilant. After exposure in a sterilizer, the glass ampoule containing media is crushed to activate the unit (allowing the spores access to the recovery media) and incubated to check for sterility.
3) BI Ampoules typically are a one (1) to four (4) ml sealed glass ampoule containing spores suspended within recovery media. After the ampoule is filled it is hermetically sealed. This type of BI requires no activation, and the transfer step associated with spore strips is eliminated. As steam condenses on the glass, the energy from the steam is transferred across the glass to the liquid media within the ampoule. Temperature and pressure within the ampoule rise, creating a micro-environment similar to that outside the Ampoule, leading to spore death. After the cycle the ampoule is incubated to check for sterility.

MATERIALS:

- Spore Strips and Prospore Ampoules manufactured by Raven Biological Laboratories, and Attest Biological Indicators for Steam manufactured by 3M. All BIs used contained B. stearothermophilus spores (ATCC # 7953). Specifications for the BIs used are available in Figure 4.
- Joslyn BIER (Biological Indicator Evaluator Resistometer) Vessel
- Amsco Based Steam Sterilizer modified to perform air removal testing by introducing a measured "LEAK" of air into the chamber.
- 16 Towel Test Packs constructed according to AAMI Good Hospital Practice: Steam Sterilization and Sterility Assurance; section 7.6.1
- Baxter Constant Temperature Oven
- Intergraph Chemical Integrators - performance specification: complete color change at 3.5 minutes @ 134^0C
- Cross Checks Chemical Integrators
- USP 23 (supplement 5) Viable Spore Count Procedure and Survival/Kill determination and calculation
- Laminar flow hood (horizontal) for strip transfer
- Modified Sterile Soybean Casein Digest Broth
- Incubator ($54\text{-}57^0$C)

METHODS:

Selection of the BIs was based on similar kill times, calculated from the manufacturer's stated population and Dvalue using the USP Survival/Kill Time Equations. This USP guideline gives the user a basis to determine if the BI is performing according to manufacturers stated label claims for population and resistance. When the BIs are exposed in a sterilizer at specific conditions for the calculated kill time, all BIs should be dead. When exposed at the calculated Survive Time, all BIs should exhibit growth. BI selection was done according to this guideline so the various BIs could be run side by side for direct comparison. All BIs were stored and cultured according to the instructions provided by the manufacturer. The following tests were performed to compare the performance of the Ampoule BI to spore strip and/or another self-contained BI:

1) Paper spore strips and Ampoule BIs were exposed in a Joslyn BIER vessel at 121^0C for 6, 8, 10 and 12 minutes. This was intended to determine if the fraction of negative BIs (those killed) varied substantially when different BIs were exposed together at various time intervals. Results shown in Figure 1a.
2) Twenty spore strips and twenty Ampoule BIs were exposed in a Baxter Dry Heat Oven at 121^0C for fifteen minutes and another twenty of each at thirty (30) minutes. This situation represents the worst case scenario for "poor steam quality" (i.e Dry Heat = no steam). The number of positive BIs for each cycle is listed in figure 2a.

3) Ampoules, spore strips, Chemical Integrators and a self-contained BI were run side by side in an autoclave equipped with a device to introduce a measured "air leak" into the chamber. This device is intended to create a definable, poor quality steam environment. Three (3) different leak sizes (0, 1L, and 2L) were introduced during two (2) different temperature cycles (121^0C and 134^0C). The three different types of BIs were directly exposed in the following conditions and then cultured.

   a) 121^0C with no leak for 15 minutes
   b) 121^0C with 1000 ml air leak for 15 minutes
   c) 134^0C with no air leak for 4 minutes
   d) 134^0C with 1000 ml air leak for 4 minutes

Results shown in Figure 3a.

4) Two each of the Ampoules, spore strips and self-contained were placed within of a 16 towel Biological Indicator test pack assembled according to AAMI guidelines. The packs were run under the following conditions and then the BIs were removed and cultured.

   a) 134^0C for 1 minute with no leak
   b) 134^0C for 2 minutes with no leak
   c) 134^0C for 3 minutes with no leak
   d) 134^0C for 3 minutes with a 1000 ml leak
   e) 134^0C for 3 minutes with a 2000 ml leak
   f) 134^0C for 4 minutes with a 2000 ml leak

Results shown in Figure 3b.

DISCUSSION:

Exposures to various cycles in the BIER vessel show the self-contained indicator and the Ampoule exhibit dichotomous responses at longer exposures than the strips. Possibly this is due to the fact that the self-contained and the Ampoule have more mass and take longer to heat up to lethal conditions.

Exposure to dry heat was not able to kill either the spore strips or the ampoules in 15 or 30-minute exposures (self-contained not exposed in Dry Heat Oven). These results indicate that both the spore strip and the ampoule are sensitive to the lack of steam, which is necessary for the rapid transfer of energy to kill the spores. Dry Heat is shown to be slower at transferring its energy than steam.

Direct exposures in an autoclave with a measured air leak show that none of the BIs tested are sensitive to the air leak when the air leak is not localized to single location (i.e. within a pack).

Results of BI exposures within AAMI packs show that at 1, 2, and 3 minutes at 134^0C with no leak, the three BIs behave identically. At 3 and 4 minutes with 1000 ml leak the BIs were insensitive to this leak, but there were two packs per cycle, so the leak may have been only partially localized within each pack. At 3 min with 2000 ml leak some positive self-contained and spore strips were seen. Again there were 2 packs per cycle, so more failures might have been seen had there only bee one pack run per cycle. The results may be due to the unit's placement within the pack. Further testing to obtain data with more statistical reliability is still needed. The cycle at 134^0C for four minutes only had one pack per cycle, so it was subjected to the entire leak, instead of being split between two packs (as in the 3 min/2L leak cycle). This led to poorer steam conditions within the pack and survival of the one self-contained, one of the Ampoules and two of the spore strips.

Conclusion

It was assumed that since all types of indicators were tested in the same manner for Dvalue (in a BIER Vessel Meeting AAMI/ANSI performance standards) and population (according to USP population determination guidelines) they should behave similarly within varying sterilization cycles. It was also assumed that of the three, the self-contained would be most sensitive to an air leak because of the product's design and ability to trap air. Spore strips and the Ampoule, though without the direct ability to capture air appear to be nearly as sensitive to adverse conditions. The data indicates that none of the BIs are sensitive to a 1L

air leak, which is detectable by typical Bowie-Dick test sheet or pack. Poor Quality Steam, achieved by introducing air into the chamber, does not appear to be readily detectable by any of the three packaging configurations. BIs are not normally expected to do so. Gross leaks of 2L appear to be detectable by each of the three types of BI packaging configurations. It appears that all three types of BIs perform adequately within a typical porous load where adequate steam penetration has taken place. All three lose sensitivity when the leak is small and divided among more than one pack. The data collected should be considered preliminary. Hundreds of each BI should be run to be able make decisive conclusions.

FIG 1a

BIs Exposed in a Joslyn BIER Vessel for various exposure lengths. Data indicates the # of BIs exposed/the # of BIs that Survived (percentage of those exposed that survived)

	6 min	8 min	10 min	12 min
Ampoule	20/20 (100%)	20/20 (100%)	20/14 (70%)	20/0 (0%)
Spore Strip	20/20 (100%)	20/10 (50%)	20/0 (0%)	20/0 (0%)
Self-contained	20/20 (100%)	20/20 (100%)	20/6 (30%)	20/0 (0%)

Fig 2a

BIs Exposed in a Dry Heat at 121^{0}C Baxter Constant Temperature Oven
Data Indicates # of BIs exposed/# Positive for growth

	15 min @ 121^{0}C	30 min @ 121^{0}C
Spore Strips	20/20	20/20
Ampoules	20/20	20/20

FIG 3a

Direct Exposure of BIs at 121 and 134 Degrees C with no Leak and with a 1000ml Leak.
Data indicates the # of BIs exposed/# of BIs positive for Growth

	Ampoule	Spore Strip	Self-Contained
15 min @ 121^{0}C No Leak	4/0	4/0	4/0
15 min @ 121^{0}C 1000 ml Leak	4/0	4/0	4/0

4 min @ 134^{0}C 1000 ml Leak	4/0	4/0	4/0

Two each of Integraph and Cross Checks Integrators were exposed with the BIs. All exposed integrators showed complete color change.

Figure 3b

BIs and CIs Placed within an AAMI defined 16 Towel Test Pack
Data indicates # of BIs Exposed/# Positive for Growth
Chemical Integrators either Pass or Fail

	Prospore Ampoule	Spore Strip	Attest Self Contained	Chemical Integrators
1 min @ 134 No Leak	4/0	4/0	4/0	Intergraph - FAIL Cross Checks - PASS
2 min @ 134 No Leak	4/0	4/0	4/0	Intergraph - FAIL Cross Checks - PASS
3 min @ 134 No Leak	4/0	4/0	4/0	Intergraph - FAIL Cross Checks - PASS

3 min @ 134 1000 ml Leak	4/0	4/0	4/0	Intergraph - FAIL Cross Checks - PASS
4 min @ 134 1000 ml Leak	4/0	4/0	4/0	Intergraph - FAIL Cross Checks - PASS
3 min @ 134 2000 ml Leak	4/0	4/1	4/3*	Intergraph - FAIL Cross Checks - PASS
4 Min @ 134 2000 ml Leak	4/1	4/2	1/1*	Intergraph - FAIL Cross Checks - PASS

* 3 minute cycle run with 2 packs in chamber (i.e. 2 liters of air split between two packs)
* 4 minute cycle run with 1 pack in chamber and only 1 self-contained BI (i.e. 2 liters of air with only one pack to absorb it).

Fig 4

Manufacturer's Label Claims and Calculated Kill Time

Spore Strip Lot # 315732

D_{121} 1.8 min
1.8×10^5 cfu
16.7 min USP Calculated Kill Time

Ampoule Lot # C-119

D_{121} 1.8 min
4.0×10^5 cfu
17.2 min USP Calculated Kill Time

Attest Lot # 2000-9AC

D_{121} 1.7 min

7.2×10^5 cfu
16.8 min USP Calculated Kill Time

USP Kill Time Equation

[log (population) + 4] X Dvalue