



Taking the "Mystery" Out of Biological Indicator Storage

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It is generally well known that biological indicators (BIs) used for monitoring sterilization processes are characterized by a specific viable spore count (population) as well as a specific D-value (resistance). Much has been written regarding the proper selection of BIs, and previous editions of Spore News have addressed the importance of D-value and Z-value, as well as Survival and Kill Times. For further discussion of these terms, please refer to the Spore News article, Sterilization Vocabulary, Vol. 10, No. 5 by Garrett Krushefski at <http://www.mesalabs.com/wp-content/uploads/2013/09/Spore-News-Vol-10-No-5.pdf>.

Attention to such characteristics as population and D-value is obviously a critical step in BI selection; however, I've discovered over the years that BI storage (which can adversely affect these characteristics) often fails to receive the attention it deserves.

I occasionally speak with customers who have inadvertently stored a biological indicator such as ProSpore Ampoule or MagnaAmp at room temperature, whereas the manufacturer's recommendations state that the ampoules should be refrigerated. Similarly, I've encountered situations where spore strips which should have been stored at room temperature were accidentally placed in a freezer. Improper storage can alter both the BI's population and the resistance, so it is important to closely follow the manufacturer's recommendations in order to assure that the biological indicator performs as intended.

While detailed storage recommendations may be found on the BI's Instructions for Use (IFU), Certificate of Analysis (CofA), the product performance data card and the product label, mistakes can occasionally be made when individuals are rushed or they receive a variety of BIs at their facility all at once. Obviously, the best rule of thumb is to always refer to the manufacturer's instructions when storing BIs, but a few basic tips may help to avoid errors.

In terms of storage, there are two important points to consider. First of all, because biological indicators are living organisms, it's always best to keep them away from sterilizing agents, chemicals, direct sunlight and all other forms of UV light. Secondly, certain types of biological indicators (e.g. ampoules) may grow prior to incubation if they experience an elevated temperature excursion; even if "optimal growth temperature" is not realized. For example, the optimal growth temperature for *G.stearothermophilus* is 55 to 62°C. However, the spores will germinate and grow (albeit slowly) at 40+°C. Therefore, it is important to heed the recommended storage conditions. Most cases of improper storage seem to be due to storing BIs at the improper temperature so perhaps some of these errors may be avoided by considering the characteristics of the BIs in the following categories.

Note: While the following information emphasizes the appropriate temperature storage range, BI storage instructions also include detailed information regarding relative humidity as well as exposure to chemicals and UV light. Please refer to Manufacturer's Recommendations when storing any biological indicator.

Ampoules and Suspensions

Refrigerate at 2 – 8°C. Biological indicators in this group consist of spores immersed in media or liquid. Since the BIs are in contact with media (which is essentially the “food” for the bacteria), the only remaining factor needed for growth is optimal growth temperature. Refrigeration at 2 – 8°C prevents these BIs from germinating.

- ProSpore Ampoule
- ProAmp
- MagnaAmp
- SterilAmp II
- SterilAmp 5230
- SterilFlex
- Suspensions



ProAMP



ProSpore



Suspensions

Note: Ethanol suspensions manufactured at Mesa’s Bozeman Manufacturing Facility should be stored in the freezer at -10 to -25°C.

Industrial Use BIs, Spore Strips and Crushable Self-Contained BIs (SCBIs)

Store at Room Temperature. This larger category includes BIs which consist of an inoculated carrier (made of paper, steel, cotton, borosilicate or sand) which is not in contact with culture media. (Most of the BIs listed below are transferred to a tube of culture media after processing.)

ProTest and EZTest are unique in that they are Crushable Self-Contained BIs which contain a spore strip or spore disc which is separate from the inner media vial until the BI is activated (“crushed”). Because the spores are not in contact with media, growth cannot occur and refrigeration is not necessary (or recommended).

Although ProSpore Ampoule listed in the first group is also considered a “Self-Contained BI” (meaning that it contains spores and media in one unit), ProSpore Ampoule contains spores immersed in culture media which places this BI in the group requiring refrigeration.

- Steel Discs/Coupons
- Steel Wires
- Cotton Threads
- Polyester Sutures
- Paper Discs
- Borosilicate Discs
- ProLine
- Spore Strips/MesaStrips
- micro Strips
- ProTest
- EZTest
- Smart-Read® EZTest
- DriAmp



Steel Coupons



EZTest



Spore Strips

Apex Products:

Store at 2 - 8°C; less than 50% RH; move to ambient conditions ≥ 1 hour before use. Because BIs stored under high humidity conditions may have an increased resistance to hydrogen peroxide, Apex BIs should be stored at less than 50% RH.



Apex Discs

- HMV-091 Log 6 *G. stearothermophilus* (#12980) stainless steel discs, packaged in Tyvek/Tyvek®
- KCD-404 Log 4 *G. stearothermophilus* (#12980) stainless steel discs, packaged in Tyvek/Tyvek®
- SBC-327 Log 6 *G. stearothermophilus* (#12980) stainless steel ribbons, packaged in Tyvek/Tyvek®
- LOG-456 Tri-Scale BI, *G. stearothermophilus* (#12980), packaged in Tyvek/Tyvek®
- GRS-090 Log 6 *B. atrophaeus* (#9372) stainless steel discs, packaged in Tyvek/Tyvek®
- NAS-152 Log 6 *G. stearothermophilus* (#7953) stainless steel discs, packaged in Tyvek/Tyvek®

Again, it’s always best to refer to the manufacturer’s recommendations when storing biological indicators. However, in the event that this isn’t possible, perhaps an explanation of “why” BIs are stored in a certain manner can take the “mystery” out of proper BI storage and eliminate unnecessary errors.

Annette joined Mesa Labs in April of 2007 as a Sales Representative where she assisted customers in the domestic health-care market. She later transitioned to Technical Support where she provided technical presentations and support to clinical and industrial BI users. Annette continues to provide technical guidance in her current role as International Sales Manager of Mesa’s BI division.