Biological indicators for hydrogen peroxide vapor technology

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Biological indicators (BIs) have become the industry standard for verification of hydrogen peroxide vapor (HPV) decontamination efficacy. This white paper discusses BIs for use with HPV and some of the factors associated with inconsistent performance of BIs used to assess HPV bio-decontamination cycles.

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Biological indicators (BIs) are defined loosely as a characterized strain and population of a microorganism that can be used to monitor the efficacy of a decontamination process. They continue to be considered the 'gold standard' as they demonstrate the ability of the chosen method to inactivate highly resistant microorganisms. The strain, preparation and presentation of the BI vary, depending on the process the BI is designed for. This article will concentrate on BIs designed for hydrogen peroxide vapor (HPV) bio-decontamination.

Issues with HPV BIs

There is an increasing issue with inconsistency in BI batches and so-called 'rogue' BIs. Batch variability in BIs leads to false positives, and although less easily detected, false negatives. Inaccurate or unexpected results when re-validating HPV cycles can potentially have huge consequences on the BI users. Impacts range from having to repeat cycles to scrapping of product and shutdown of facilities, all of which could pose a significant financial impact upon the user.

As a result of these issues, a huge importance has been placed on the quality and repeatability of BIs for use with HPV in particular.

Factors affecting resistance to HPV

Several factors have been observed to affect the resistance of a BI to inactivation by HPV technology. Some are related to the microbial strain and the preparation of the spores, others are a reflection of the type and quality of the carrier material. The following explores some of the major factors affecting BI resistance to HPV.

Spore preparation

Spores inoculated onto carriers should be produced from a recognized type culture strain known to be particularly resistant to HPV.[1-3] The microorganism should be more resistant than most other microorganisms, should be safe to use and provide a consistent challenge to the HPV process. *Geobacillus stearothermophilus* endospores are most commonly used to prepare BIs for the HPV process e.g. strain ATCC 12980 or ATCC 7953.[1-3]

The spore preparation used to produce BIs should be free of contaminants and should consist predominantly of endospores. However, scanning electron micrograph (SEM) images show many of the HPV BIs currently available have issues with contaminating materials (Image 1). Media, cell debris, salts and other contaminants reduce the efficacy of HPV penetration and can lead to inconsistent and unexpected results during cycles, typically observed as false positives. BIs prepared with large numbers of vegetative (non-sporulated) microbial cells present are likely to be a cause of false negative results due to the lower resistance of vegetative cells compared to their spore counterparts.

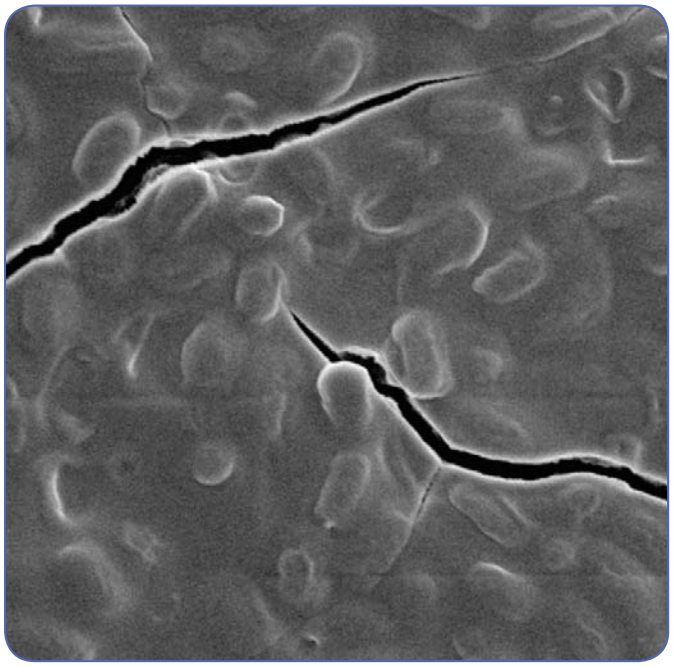

Image 1. *G. stearothermophilus* spores encased in thick media components on a commercially available BI.

Carrier material and finish

The carrier material used for production of BIs should be carefully chosen to reflect the types of materials being decontaminated and also the process being applied. HPV BIs require a carrier that is not absorptive. Materials that have been suggested for use are metals (stainless steel, aluminium, etc), glass, plastic and ceramic.[1] Most carriers for HPV processes use stainless steel as it is representative of many commonly decontaminated surfaces (e.g. in isolators) and has been shown to be relatively inert when in contact with HPV.

Another important consideration is the surface finish of the carrier. Uneven or pitted surfaces (Image 2) can potentially protect spores from the effects of HPV and lead to inconsistency in the performance of the BIs resulting in false positives.

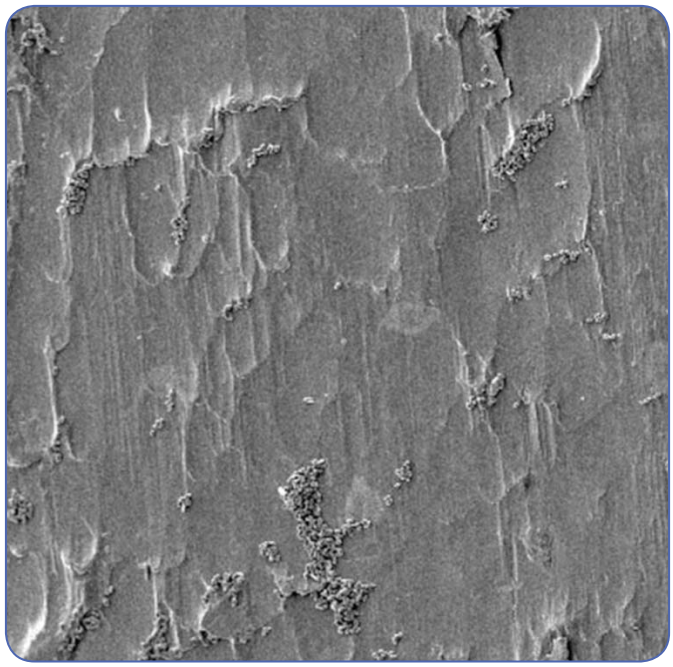

Image 2. Uneven surface finish of carrier disc causing formation of spore aggregates on a commercially available BI.

Spore distribution

The distribution of spores across the chosen carrier material is also important to consider as aggregates or dense layers of spores can offer protection to spores either in the center or underneath respectively (Image 3 and 4). Again this can lead to false positives. Factors affecting the distribution of spores include (but are not limited to) the carrier type/finish, carrier shape, bacterial suspension components, drying method and bacterial suspension volume (loading).[1] Image 5 shows ideal spore distribution and cleanliness.

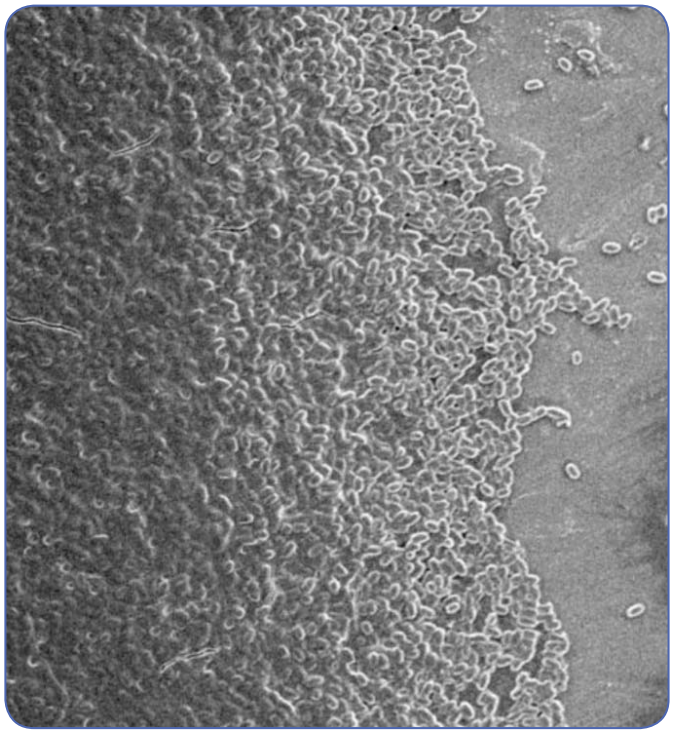

Image 3. Areas of dense microbial load on a commercially available BI.

Image 4. Areas of dense and uneven microbial load (several layers thick) on a commercially available BI.

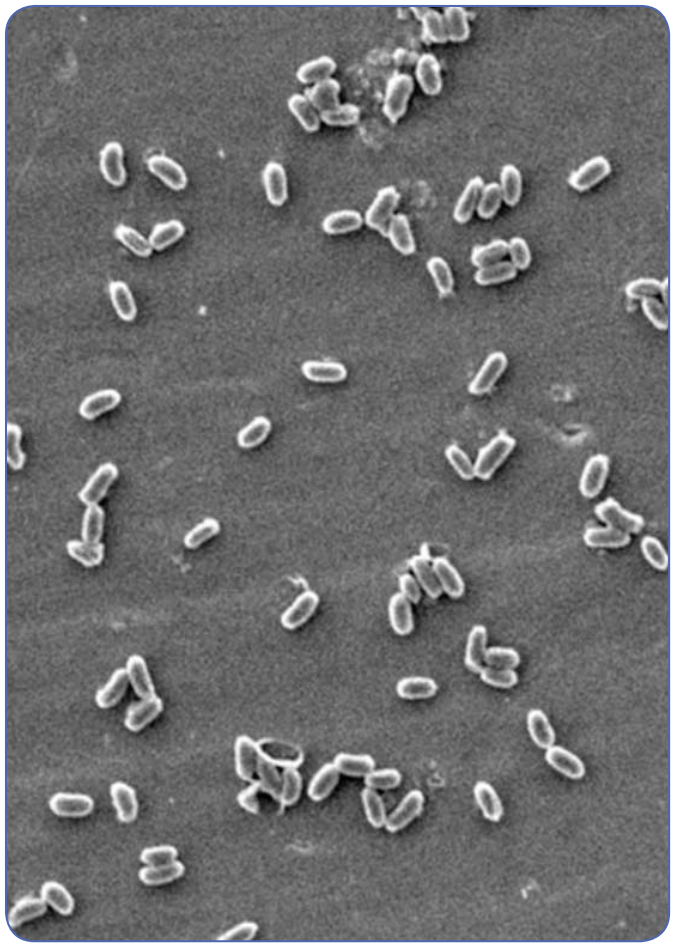

Image 5. Even distribution of clean *G. stearothermophilus* spores across a stainless steel carrier disc.

Biological indicator resistance characteristics

Decimal reduction time (D-value) is defined as the time required for reduction of 90% or 1-log of a microorganism upon exposure to decontaminating conditions. D-value is used as a measure of the relative resistance of the BI and is an important consideration when using BIs for validation, re-validation and cycle development. For example, using a BI with a D-value of 1 minute at initial validation followed by a BI with a D-value of 2.5 minutes at re-validation could potentially lead to cycle failure.

It is therefore important that the BIs used for these purposes are pre-qualified and found to be within an acceptable limit for the user. D-value is often called 'system D-value' as there is no currently accepted resistometer or BIER vessel available for HPV resistance testing and as such the D-value stated is specific to the equipment and methodology used.[1]

Conclusion

This white paper highlights some of the sources of variation and potential inconsistency with BIs used to assess HPV bio-decontamination cycles. It is important that users understand the inherent variability of BIs and perform suitable quality control procedures to ensure consistent performance in the field.

Reference

1. PDA Technical Report No.51 (2010) Biological Indicators for Gas and Vapor-Phase Decontamination Processes: Specification, Manufacture, Control and Use
2. ISO 11138-1(2006) Sterilization of health care products -- Biological indicators -- Part 1: General requirements.
3. United States Pharmacopoeia (2005) General chapter: Biological indicators for sterilization.

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