SporeNews
biological indicators newsletter
MesaLabs
Volume 13, No. 3

# Biological Indicators and the European Pharmacopoeia

by Nicole Robichaud

Mesa Labs manufactures biological indicators (BIs) in compliance with the United States Pharmacopeia (USP) and the ISO 11138 series, Sterilization of Health Care Products – Biological Indicators. On August 1, 2016, a new revision of the USP was made available which included significant changes to the general chapters and monographs pertaining to BIs, as detailed in Spore News Volume 13, No. 1. In February 2017, a revision to the ISO 11138 series was published and the changes were outlined in Spore News Volume 13, No. 2.

A new revision of the European Pharmacopoeia (EP) General Chapter 5.1.2, concerning BIs, was published July 1, 2017. Although Mesa Labs' did not previously claim compliance to the EP, many of our international customers do and for this reason, this Spore News article will focus on the new revision EP 9.2, 5.1.2.

The previous version of EP 5.1.2 was concise compared to the new revision and consisted of only five sections. The first section provided a general description of BIs, BI characterization by organism and D-value, placement of BIs in a sterilisation load and choice of indicator organisms for BIs. The next four sections each pertained to specific sterilisation processes, Steam sterilisation, Dry-heat sterilisation, Ionising radiation sterilisation and Gas sterilisation. Each of these sections provided pertinent information such as the organism recommended for the sterilisation process and the recommended minimum BI population and D-value for each process.

The new revision of EP 5.1.2 consists of six sections and each section contains many subsections; the information is more detailed and covers a wider scope than the previous version. A summary of each section is provided below.

**Title**

- The title was made more descriptive by being changed from Biological Indicators of Sterilisation to Biological Indicators and Related Microbial Preparations used in the Manufacture of Sterile Products.

**1. Introduction**

- Describes BIs and their intended use.
- Introduces the concept of using reduced sterilisation process conditions to ensure the validity of the process.
- Directs that no surviving organisms should be observed when the BI is subjected to the full sterilisation process.

**2. Biological Indicators for Sterilisation Processes**

- Factors influencing sterilisation process effectiveness.
- Describes how physical validation demonstrates that process conditions are delivered homogeneously to all parts and positions of the load.
- Describes how biological validation demonstrates the correlation between the predicted effect of the physical conditions and the observed effect on the BIs.
- Factors influencing BI selection.
- Directs that the most difficult to sterilise position in the load and product must be determined.
- Explains that spores directly inoculated into or onto product will react differently than BIs to the sterilisation process.

- Instructs that the end-user can rely on the BI manufacturer's label claims if they have established a high level of confidence in the BI manufacturers compliance to quality standards through auditing or the label claims shall be verified.
- Defines of four types of BIs, inoculated carriers, self-contained BIs, spore suspensions and custom-made BIs.
- Directs that the D-value and z-value, where appropriate, of custom-made BIs (inoculated product or items) must be determined.
- Quality requirements for BIs (information the end user must know for each lot).
- User requirement specification (URS) for BIs.
- BI Quality Control activities for end-user – compliance with URS, purity, visual identification and viable count.

**3. Biological Indicators for Heat Sterilisation**

- Defines z-value, Survival Time and Kill Time.
- Describes how a to establish a validation cycle which demonstrates the correlation between the predicted effect of the physical conditions and the observed effect on the BIs by use of reduced sterilisation conditions.
- Recommends BI organism, typical population and typical resistance of BIs for moist heat sterilisation and dry heat sterilisation.
- Recognizes that Geobacillus stearothermophilus may not be appropriate for moist heat processes delivering an F0 between 8 and 15.

**4. Biological Indicators for Gas Sterilisation**

- Directs that BIs are necessary for all gaseous sterilisation development, validation and monitoring.
- Recommends BI organism and population of BIs for Ethylene Oxide sterilisation.
- States that BI organism must provide D-values relevant to the process being validated but does not provide typical resistance.
- Recommends BI organism for vaporized hydrogen peroxide processes.
- Clarifies that gas surface disinfection is outside of the scope of the EP.
- States that it is the responsibility of the user to define the sterilisation cycle and the suitability of the BI used for gas sterilization processes other than Ethylene Oxide.

**5. Biological Indicators for Ionising Radiation Sterilisation**

- Recommends BI organism.
- States that for validation of the sterilizing dose, BIs are generally not considered necessary but in certain instances BIs may be required for the development and validation of ionizing radiation sterilisation.

**6. Microbial Preparations for Sterilisation Grade Filtration**

- Describes how to validate a membrane filtration sterilisation process.
- Recommends organisms to use for the validation of filter retention.

In 2014, Spore News Volume 8, No. 2 titled The Problem with EP 7.0, 5.1.2, was released. In this article, the author identified three EP requirements that did not make sense when considering the EP recommended BI resistance in place at that time. We are quite pleased to see that none of the three appear in the new revision of EP 9.2, 5.1.2.

Additionally, it has been verified that Mesa Labs' BIs now comply with the EP 9.2, 5.1.2; however, three of the quality requirements listed in section 2.2 are not reported on documents provided to the end-user, Certificate of Analysis, Technical Report or Instructions for Use.

Two of the requirements are the 95% confidence interval and the number of replicates tested for D-value determination. The D-value of Mesa Labs' BIs is determined per ISO 11138-1 which specifies the number of BI replicates used for each method of resistance determination and specifies calculation of the 95% confidence interval when using the Limited Holcomb-Spearman-Karber procedure. Mesa Labs' approved procedure for resistance determination also specify these details.

The third requirement is reporting the range of temperatures used to determine z-value. Like D-value, the z-value of Mesa Labs' BIs is determined either per ISO 11138-3 Biological Indicators for Moist Heat Sterilization Processes or per ISO 11138-4 Biological Indicators for Dry Heat Sterilization Processes. Both ISO documents specify the temperature range used for z-value calculation for each process. The temperatures used to calculate z-values, which are within the ranges specified by ISO, are also included in Mesa Labs' approved procedure for resistance determination.

There are two items in the new revision of EP 5.1.2 that are worth noting;

The first item is the somewhat contradictory recommendations for end-user verification of BIs. Section 2-1 DESCRIPTION OF BIOLOGICAL INDICATORS FOR STERILISATION PROCESSES states, "The user must establish a high level of confidence in the manufacturer's compliance to quality standards for the biological indicator (e.g. by means of auditing) in order to rely on the characteristics stated by the manufacturer (see section 2-2). Alternatively, the labelled characteristics of biological indicators shall be verified by the user or by an independent, contract laboratory that is formally approved by the user." Section 2-2-2 Quality control states, "Quality control for biological indicators consists of testing for purity, identity and estimation of the number of viable cells." The statement in section 2-1 leads one to believe that if audits are not performed, the end-user should verify all labeled characteristics of the BI, identity, purity, population, D-value and Z-value, whereas the statement in section 2-2-2 suggests the end-user need only test BI purity, identification and viable count.

The second item is the following statement contained in the INTRODUCTION, "Biological indicators are intended for the development and validation of the sterilisation processes and not for routine monitoring unless otherwise stated in this general chapter." Mesa Labs manufactures BIs that are appropriate for and intended to be used for development, validation and routine monitoring of sterilisation cycles and supports the use of BIs in all sterilisation cycles. This may sound suspect coming from a BI manufacturer; however, BIs are the only tool capable of integrating and responding to all critical process parameters. We firmly believe that use of a BI to monitor routine cycles provides a relatively inexpensive insurance for sterility assurance, whereas omission of the BI unnecessarily increases one's risk to not detect a cycle failure.

*Nicole Robichaud graduated from Montana State University with a B.S. in Biological Sciences. Nicole has worked for Mesa Labs since 2007 and has held positions in the Spore Cultivation Laboratory, Quality Control and currently is a Scientific and Technical Service Representative.*