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Biological Indicators and the United States Pharmacopeia

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Mesa Labs manufactures its biological indicators (BIs) in accordance with several standards. In a previous edition of Spore News, we discussed the AAMI/ISO standards which pertain to BIs manufactured for the medical device industry (Spore News Vol 12, no 1). This edition covers BIs manufactured for use in the pharmaceutical industry as detailed in the United States Pharmacopeia (USP). As is the case with any standard that is designed to remain relevant, the USP undergoes periodic revisions. The most recent revision of the USP contains significant changes pertaining to biological indicators and their use in the industry and these will be discussed in this Spore News edition.

A Brief History of the USP

The USP was established on January 1, 1820, in the senate chamber of the U.S. capital building. Its purpose was to create a compendium of therapeutic products and provide recipes for their preparation. A comprehensive timeline of changes to the organization can be found on the USP website1 however a few dates of interest include:

- 1830 - a Committee of Revision was created and the USP is revised at 10-year intervals.
- 1900 - USP incorporated as a not-for-profit corporation and the Board of Trustees is created.
- 1938 - The Federal Food, Drug, and Cosmetic Act passed and the USP is recognized as official and is enforced by the FDA.
- 1942 - The USP revision cycle is changed from every 10 years to every 5 years.
- 2002 - The USP is revised and published annually.

Revisions now occur continuously and are presented annually in the USP and twice-annually as Supplements to the USP.

USP Structure

The USP is organized into numerous chapters. General chapters numbered 1 to 999 (i.e. monographs) are considered "enforceable" whereas general chapters numbered greater than 1000 are for informational purposes only.

In the current USP revision, BI content is predominantly in chapters <55> *"Biological Indicators—Resistance Performance Tests"* and <1229.5> *Biological Indicators for Sterilization"*, although most chapters in the <1229> series also contain BI references.

BIs and the USP

Biological Indicators are not pharmaceutical products so why are they included in the USP? The USP mission statement, *"To improve global health through public standards and related programs that help ensure the quality, safety, and benefit of medicines and foods"*, provides the answer. In short, BIs are a tool necessary for assuring the "quality" and "safety" of pharmaceutical products and as such, guidance for their preparation and use are included in the USP.

Chapter <55>, considered enforceable, describes the methods and materials used to determine the resistance of BIs and is intended for both the manufacturer and the user. It sets tighter controls for the manufacturers on the use of replicate units per test, while stating "the end users are not required to use the same number of replicates for verification of those determinations".

A comparison of differences between the current revision and the previous version of chapter <55> is presented in Appendix 1. The most significant change is that all BI information has been deleted from the official monographs section and much of it relocated in chapter <55>. This table is not all inclusive and the reader should refer to the USP for complete details.

New informational chapters to the USP are numbered in the <1229> series and largely replace the deleted chapter <1035> "Biological Indicators for Sterilization". This series provides a well written and easy to understand description of the basic principles for control of a sterilization process including, process development, process validation, and maintenance/monitoring.

As Biological Indicators are tools that are used in each of these steps, they are discussed throughout most of the <1229> series which include:

- <1229> Sterilization of Compendial Articles
- <1229.1> Steam Sterilization by Direct Contact
- <1229.2> Moist Heat Sterilization of Aqueous Liquids
- <1229.3> Monitoring of Bioburden
- <1229.4> Sterilizing Filtration of Liquids
- <1229.5> Biological Indicators for Sterilization
- <1229.6> Liquid Phase Sterilization
- <1229.7> Gaseous Sterilization
- <1229.8> Dry Heat Sterilization
- <1229.9> Physicochemical Integrators and Indicators for Sterilization
- <1229.10> Radiation Sterilization
- <1229.11> Vapor Phase Sterilization
- <1229.12> New Sterilization Methods

A comparison between the <1229> chapters and the deleted chapter <1035> is presented in Appendix 2. This table is not all inclusive and the reader should refer to the USP for complete details.

Discussion

It is our opinion that the latest revision of the USP provides significant improvements in both its organization and presentation of the Biological Indicator subject matter. Some of the values previously presented in the general information chapter (e.g. the D-value ranges in chapter <1035>) were interpreted by many firms to be enforceable, and as such caused some confusion. These have been removed in the latest revision. Additionally, the USP increasingly references existing standards that meet their expectations (e.g. ISO 11138 series-Biological Indicators, and ISO 18472-Resistometers), rather than create a redundant set of standards.

Appendix 1. Comparison of Current Revision of Chapter <55> to Deleted Enforceable Chapters

Subject	Pre-August 1, 2016 Version	Post August 1, 2016 Version
Population – Number of Samples	**Chapter <55>** • three specimens	**Chapter <55>** • manufacturer must use "at least four test samples"
Population - method	**Chapter <55>** • blender cup method.	**Chapter <55>** • does not specify method, only states to "mechanically disrupt to achieve a homogeneous suspension"
Population – test tube size	**Chapter <55>** • screw capped 16 x 125 mm	• Not specified
Population – acceptable range for verification of labeled population	**USP Monographs** • For paper carriers and self-contained: "log average number of viable spores per carrier is not less than 0.3 log of the labeled spore count per carrier and does not exceed the log labeled spore count per carrier by 0.48" • For nonpaper carriers: "the average number of viable spores per carrier are within -50% and +300% of the labeled count per carrier" • For liquid spore suspensions: "within ±1 log of the value stipulated by the manufacturer"	**Chapter <55>** • For vendor supplied BIs: "between 50% and 300% of the manufacturer's stated value" • For spore Suspensions, "within 50% - 300% of the labeled count"
Population – plate counts	**Chapter <55>** • "preferably 30 to 300 colonies, but not less than 6, on each of a pair of plates".	**Chapter <55>** • "30 to 300 colonies on each plate in a pair".
D-value – Number of Samples	**Chapter <55>** • "Take a sufficient number of groups of specimens of biological indicators in their original individual containers, each group consisting of 5 to 10 specimens."	**Chapter <55>** • "Use 20 replicate test sample BIs in their original individual containers, subjected to at least five exposure conditions for a total of 100 tests."
D-value – Verification	**Official Monographs** • "The requirement of the test are met if the determined D value is within 20% of the labeled D value…"	**Chapter <55>** • Not specified in this chapter
Purity	**Official Monographs** • "Purity: By examination of the colonies derived from the spores on a suitable plate culture medium, determine that there is no evidence of contamination with other microorganisms."	**Chapter <55>** • Not specified in this chapter
Survival/Kill	**Official Monographs** • Equations for calculation of Survival and Kill times • Acceptance criteria • Retest procedure if not more than one BI fails the procedure described in Chapter <55> **Chapter <55>** • Procedure for verification of values per the equations presented in the official monographs	**Chapter <55>** • ". . . should be provided by the manufacturer and verified by the end user."

Appendix 2. Comparison of Current Informational Chapter <1229> to Deleted Chapter <1035>

Heading	General Chapter <1035> Biological Indicators for Sterilization	General Chapter <1229.5> Biological Indicators for Sterilization
Introduction/Proper Use of Biological Indicators	• General description of a BI, the organisms used for BIs and the applications of BIs	• General description of a BI, the organisms used for BIs and the applications of BIs
BI Manufacturer's Responsibility	To provide: • Certificate of Analysis • Microbial count and resistance stability throughout shelf-life • D-value and method by which it was determined • Storage conditions • Directions for use, including medium and conditions used for recovery • Disposal instructions	To provide: • Certificate of Analysis • Microbial population • Resistance (D and Z values where appropriate) • Storage conditions • Expiry • Directions for use, including medium and conditions used for recovery • Disposal instructions
BI User's Responsibility	• Should establish suitability for use in a specific sterilization process • Should establish in-house acceptance standards for BIs • Should obtain a Certificate of Performance • Should perform audits of manufacturer • Should verify purity and morphology • Microbial count should be determined • Manufacturer's D-value range, storage conditions, expiration and stability should be observed	• Suitability for use in a specific sterilization process must be established • Should obtain a certificate of analysis • Should verify labeled population • Resistance need not be reconfirmed when used according to manufacturer's directions
User-Prepared Biological Indicators	• Users responsibility to ensure performance requirements met.	• Users responsibility to determine population and resistance.
Characterization of Biological Indicators	• Information located throughout various sections.	End user acceptance and control of BIs • Packaging and Storage • Expiration Date • Identification • Purity • Disposal
Types of Biological Indicators	General descriptions • Spores inoculated onto a carrier in a package • Spore suspension inoculated onto or into product • Self-contained, growth medium in direct contact with spores during sterilization • Self-contained, growth medium placed in direct contact with spores after sterilization	General descriptions • Spores inoculated onto a carrier in a package • Spore suspension inoculated onto or into product • Self-contained, growth medium in direct contact with spores during sterilization • Self-contained, growth medium placed in direct contact with spores after sterilization
Selection for a Specific Process	• Gives general description sterilization modalities, appropriate test organisms, and their typical resistance characteristics.	• Gives general description of sterilization modalities, appropriate test organisms, and directs reader to USP chapters <1229.1 to 1229.11> for further information.

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