MesaLabs

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Biological Indicator Selection

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With the many types of biological indicators (BIs) available today - spore strips, self-contained biological indicators (SCBIs), glass ampoule liquid submersible BIs, stainless steel BIs and Industrial Use BIs such as threads, sutures, microstrips and paper discs - the process of selecting the appropriate BI to monitor a sterilization process may seem like a daunting task. With a quick evaluation of the load type and/or the sterilization parameters however, the selection can be easily narrowed down. This Spore News will provide information about how to begin the process of BI selection, enabling end-users to more accurately determine which type of BI is appropriate for monitoring unique sterilization cycles.

Steam

The simplest way to begin the process of steam BI selection is by looking at the type of items being sterilized.

Porous or Hard Goods

If the load consists of porous or hard goods, Mesa Labs recommends using spore strips or an SCBI. Porous and hard goods are sterilized by direct contact with the steam generated by the autoclave.(1) The steam generated by the autoclave surrounds or penetrates the items and inactivates the bioburden. This is also true with the spore strips or SCBIs that would be used in autoclave loads containing porous or hard goods, steam surrounds and penetrates the BI and inactivates the spores on the BI.

Steam spore strips and steam SCBIs will function in essentially the same manner during a sterilization cycle; however, post sterilization, spore strips need to be aseptically cultured into growth media and if not handled properly, this can result in contamination of the spore strip or culture media producing a false positive result. Using a SCBI may provide an alternative option for customers who do not have the capability to perform aseptic post-process culturing as the system is 'self-contained'. Customers sterilizing items in dental offices, small clinics or body art studios may also choose to use a mail in service such as Mesa Labs Spore Testing. For more information about Mesa Labs Spore Testing see our website at www.sporetesting.mesalabs.com.

If the BI must be placed into a small area, such as tubing or a medical device, then a regular sized spore strip or SCBI may be too large and instead a small industrial use BI such as a microstrip, a small paper disc, a suture or ProLine can be used. For more information about Industrial Use BIs, see Spore News Volume 9, Number 6: Applications of Industrial Use BIs and the videos Industrial Use Biological Indicators and Biological Indicators for Validating the Sterilization of Tubing located at http://biologicalindicators.mesalabs.com/videos/.

When sterilizing medical devices, you must consider how the product and packaging effect the resistance of the biological indicator spores.[2] If the product and/or packaging system have no effect on the resistance of the BI spores, then a commercially prepared BI can be used; however, if the product and/or packaging increases the resistance of the BI spores, then a spore suspension must be used to directly inoculate the product. For more information about this topic see Spore News Volume 1 Number 3: Product D-Value Studies: A Critical Tool When Developing a Sterilization Process and Spore News Volume 12 Number 3: Contract Studies.

Containers of Aqueous Liquids

If the load consists of containers of aqueous liquid, Mesa Labs recommends one of our glass ampoule, liquid submersible BIs. Liquids are not sterilized by direct contact with the autoclave steam because the steam cannot reach the liquid inside of the container. In simple terms, the steam from the autoclave heats the container, which in turn heats the liquid contents, thus inactivating bioburden.[3] Likewise, the media inside of a liquid submersible BI will be heated and create a steam environment inside of the glass ampoule which inactivates the spores. The volume of liquid inside of the containers being sterilized will determine which liquid submersible BI to use.

For volumes of liquid less than 150 mL, Mesa Labs recommends the use of SterilAmp II. Volumes under 150 mL will heat consistently throughout with minimal to no lag time for the geometric center of the liquid to heat to sterilization temperature. SterilAmp II is a small (26 x 6.5 mm or 18 x 6.5 mm) glass ampoule that will float on top of the liquid but since there is minimal lag time for the geometric center of volumes of liquid less than 150 mL to heat to temperature, SterilAmp II will adequately monitor the conditions that are experienced by the liquid.

For volumes of liquid greater than 150 mL, Mesa Labs recommends the use of MagnaAmp or ProSpore 1 mL. Volumes of liquid greater than 150 mL will experience a lag time to temperature in the geometric center of the liquid making this the most difficult to sterilize area and because of this, the BI must be suspended in this location. MagnaAmp and ProSpore 1 mL have a neck where a fine gauge wire can be easily attached to suspend the ampoule in the liquid where it can monitor this most difficult to sterilize location.

In some instances, the liquid BI may not need to be placed into the liquid but rather can be placed at various locations in the autoclave next to the containers of liquid. Generally, Mesa Labs ProSpore 4 mL is used in this manner when sterilizing vials of product that are approximately the same volume as the ProSpore 4 mL. In this way, the ProSpore 4 mL will function as a surrogate vial and will experience the same conditions as the product vials.

For more information about liquid submersible BIs see Spore News Volume 7 Number 5: Steam Sterilization of Liquid Filled Containers and also the video Biological Indicators for Liquid Steam Sterilization located on the video page of Mesa Labs Biological Indicator website.

When sterilizing liquid pharmaceutical products and medical devices, the effect of the product and packaging on the resistance of the biological indicator spores must be considered.[3] If the product and/or packaging system have no effect on the resistance of the BI spores, then a commercially prepared liquid submersible BI can be used; however, if the product and/or packaging substantially increases the resistance of the BI spores, then a spore suspension must be used to directly inoculate the product. For more information about this topic see Spore News Volume 1 Number 3: Product D-Value Studies: A Critical Tool When Developing a Sterilization Process and Spore News Volume 12 Number 3: Contract Studies.

Containers of Heat Sensitive Liquids

The organism of choice for steam sterilization cycles at temperatures of 121 ^{o}C or greater is *Geobacillus stearothermophilus* (ATCC 7953). For sterilization temperatures of less than 121 ^{o}C which are typically used for the sterilization of heat sensitive aqueous liquid products, *Geobacillus stearothermophilus* (ATCC 7953) may be too resistant and *Bacillus subtilis 5230* (ATCC 35021) may need to be used.[4][5] For this purpose, Mesa Labs recommends SterilAmp II 5230 which utilizes the same ampoule as SterilAmp II but contains *Bacillus subtilis 5230*. For more information about SterilAmp II 5230, see Spore News Volume 1, Number 2: Biological Indicator for Monitoring Low Temperature Steam Sterilization and also Spore News Volume, 10 Number 1: Organisms and Their Uses in Biological Indicators.

Heat Sensitive Hard Goods

As with heat sensitive liquids, *Geobacillus stearothermophilus* (7953) may be too resistant for the lower sterilization temperatures used for sterilizing heat sensitive hard goods and so *Bacillus subtilis 5230* (ATCC 35021)

may need to be used.[4][5] For this application, Mesa Labs recommends the use of our *Bacillus subtilis 5230* MesaStrip, Spore Strip or microstrip if a regular sized strip is too large for the location. For more information see Spore News Volume, 10 Number 1: Organisms and Their Uses in Biological Indicators.

Waste
If the load consists of bags or containers of biohazardous waste, Mesa Labs recommends the use of one of our glass ampoule, liquid submersible BIs MagnaAmp or ProSpore 1 mL. Fluids inside of the bags or containers will coat and damage a spore strip or SCBI leaving the possibility of a false positive result but a glass ampoule BI will not be affected by waste fluids. Near the bottom of the waste will typically be the most difficult to sterilize area and so a fine gauge wire can be attached to the neck of the MagnaAmp or ProSpore 1 mL for ease of placement into the waste and retrieval of the BI after completion of the cycle. For more information about waste sterilization see Spore News Volume 9 Number 2: Medical and Lab Waste Monitoring.

Ethylene Oxide
Ethylene Oxide (EtO) sterilization is generally used for items that would be damaged by heat or radiation such as certain medical devices.[6] BI selection for this process can be less complicated than Steam BI selection simply because there is less of a variety of items that are sterilized by EtO than by steam. For EtO sterilization Mesa Labs recommends the use of EtO spore strips or SCBIs.

EtO spore strips and EtO SCBIs will function in essentially the same manner during a sterilization cycle; however, post sterilization, spore strips need to be aseptically cultured into growth media. If not handled properly post process, this can result in contamination of the spore strip or culture media producing a false positive result. Using a SCBI may provide an alternative option for customers who do not have the capability to perform aseptic post-process culturing as the system is 'self-contained'. Customers sterilizing items in dental offices, small clinics or body art studios may also choose to use a mail in service such as Mesa Labs Spore Testing. For more information about Mesa Labs Spore Testing see our website at www.sporetesting.mesalabs.com.

If the BI must be placed into a small area, such as tubing or a medical device, then a regular sized spore strip or SCBI may be too large and instead a small industrial use BI such as a microstrip, a small paper disc, a thread or a suture can be used. For more information about Industrial Use BIs, see Spore News Volume 9, Number 6: Applications of Industrial Use BIs and the videos Industrial Use Biological Indicators and Biological Indicators for Validating the Sterilization of Tubing located at http://biologicalindicators.mesalabs.com/videos/.

In some cases it may be very time consuming and costly to retrieve BIs that are embedded in large sterilization loads. After performing the appropriate validation activities with BIs embedded in the load, the user may consider using a Process Challenge Device (PCD) for routine monitoring. A PCD is selected that provides an equal or greater challenge to the cycle as compared to the embedded BI.[7] The PCDs are then placed on the outside of the load making placement and removal an easy task. For more information about PCDs, see Spore News Volume 12, Number 2: Process Challenge Devices.

Dry Heat
Dry heat is used to sterilize items that will not be damaged by heat (such as glass or stainless steel) or items that cannot be sterilized by steam (such as non-aqueous liquids or powders).[8] The main consideration for dry heat BI selection is the temperature of the cycle.

For cycles operating below 170 °C, Mesa Labs recommends spore strips inoculated with *Bacillus atrophaeus* (ATCC 9372). Both of these spore strips are packaged in a protective glassine envelope and at temperatures above 170 °C, the glue that holds the glassine envelope together can separate, leaving the strip susceptible to post process contamination.

For cycles operating above 170 °C, Mesa Labs recommends the glass ampoule DriAmp or Steel Coupons. DriAmp and Steel Coupons can withstand temperatures in excess of 350 °C and so can be used when it is necessary for a user to monitor sterilization in a depyrogenation cycle.

For low temperature dry heat sterilization of non-aqueous products such as oils, Mesa Labs recommends the use of DriAmp. Just like aqueous liquids, the conditions of the product inside of the container must be monitored. DriAmp consists of inoculated sand inside of a glass ampoule and this design will facilitate the BI being placed into the product where a spore strip would be compromised. For more information, see Spore News Volume 12, Number 4: Product Profile: DriAmp Biological Indicator.

Formaldehyde
Mesa Labs manufactures a MesaStrip and a Spore Strip in compliance with ISO 11138-5 that are specifically intended to be used in a Low Temperature Steam and Formaldehyde (LTSF) process ". . . incorporating forced air removal, which allows exposure of wrapped goods to steam at sub-atmospheric pressure, and thus at temperature < 100 °C, with the admission of formaldehyde gas, keeping the sterilizing agent in a steady state throughout the hold time."[9]

LTSF process should not be confused with Formaldehyde fumigation at atmospheric pressure which is often used for Biosafety Cabinet Decontamination. For Formaldehyde fumigation at atmospheric pressure, Mesa Labs recommends the MesaStrip or Spore Strip inoculated with *Bacillus atrophaeus* spores which are also used for EtO and dry heat sterilization.[10]

Hydrogen Peroxide
There are no specific guiding standards for hydrogen peroxide BIs but the USP provides general guidelines such as that *Geobacillus stearothermophilus* is the most prevalently used organism, the carrier should be gas-impervious and that hydrogen peroxide will degrade cellulose based materials.[5]

If a hydrogen peroxide sterilizer is being used, Mesa Labs recommends one of our SCBIs for hydrogen peroxide.

If performing vapor phase hydrogen peroxide decontamination at atmospheric pressure, Mesa Labs recommends the use of our stainless steel Apex line of BIs. The end-user should determine the appropriate BI resistance for their system since there is no standardized equipment available for vapor phase hydrogen peroxide.[11] For more information, see Spore News Volume 9, Number 3: Parameters Effecting Vapor Hydrogen Peroxide BI Performance, Spore News Volume 9, Number 4: Using Replicate BIs to Evaluate Biodecontamination Cycles in Isolators and Spore News Volume 9, Number 5: End User – Proper BI Placement During VHP Decontamination Cycles.

Alternative Sterilization Methods
There are many alternative vapors and gases which are now being utilized in the sterilization of products and enclosure surfaces. Though resistance and population requirements for processes such as peracetic acid, glutaraldehyde, chlorine dioxide, Ozone etc. are not referenced in any standards, Mesa Labs is certain to have a biological indicator solution for your needs. Frequently, customers will choose to purchase products from our Apex product line as the stainless steel carriers serve as a surrogate surface for products and enclosures. Please contact our BI Technical Support Team at BI-support@mesalabs.com for suggestions when using these types of processes.

If you have a unique situation for which one of the aforementioned BIs will not work, contact Mesa Labs and we may be able to design a custom BI to fit your needs. For additional information, see Spore News Volume 8, Number 5: Custom Biological Indicators.

While identifying the correct type of BI for a specific process should be the initial step, there are other important variables to consider. What spore population do your require? For steam sterilization, United States Healthcare is required to use a BI with a spore population of at least 105 and generally speaking, outside of US Healthcare, a BI with a spore population of 106 is used. Do you need a BI with FDA 510K clearance? United States Healthcare must use a BI with FDA 510K clearance when reprocessing items for patient use. If you are unsure about requirements you must follow, it's best to contact your regulatory agency for guidance.

All specific biological indicators mentioned above can be found on Mesa Labs Biological Indicator website at www.biologicalindicators.mesalabs.com.

Reference:

[1] USP 39 – NF 34 1229.1 Steam Sterilization by Direct Contact

[2] ANSI/AMMI/ISO 17665-1 Sterilization of health care products-Moist heat-Part 1: Requirements for the development, validation and routine control of a sterilization process for medical devices

[3] USP 39 – NF 34 1229.2 Moist Heat Sterilization of Aqueous Liquids

[4] ANSI/AAMI/ISO 11138-3:2006/(R) 2010 Sterilization of health care products-Biological indicators-Part 3: Biological indicators for moist heat sterilization processes

[5] USP 39 – NF 34 General Chapters: <1035> Biological Indicators for Sterilization

[6] USP 39 – NF 34 1229.7 Gaseous Sterilization

[7] ANSI/AMMI/ISO 14161:2009 Sterilization of health care products-Biological indicators-Guidance for the selection, use and interpretation of results

[8] USP 39 – NF 34 1229.8 Dry Heat Sterilization

[9] ANSI/AAMI/ISO 11138-5:2006/(R) 2010 Sterilization of health care products-Biological indicators-Part 5: Biological indicators for low-temperature steam and formaldehyde sterilization processes

[10] NSF/ANSI Standard 49: Biosafety Cabinetry Certification

[11] USP 39 – NF 34 1229.11 Vapor Phase Sterilization

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