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Spore News

# Sub-culturing a Positive Biological Indicator for Identification

When a positive biological indicator (BI) result is obtained from a sterilization cycle, guidance provided by ISO and the USP recommends identifying the organism responsible for the positive result. This allows the investigation to be focused on the correct area. If the organism is identified as the BI organism, the investigation should focus on the sterilization process, including but not limited to the cycle itself, the sterilizer, the physical monitors, and the load. If the organism is not identified as the BI organism, post process contamination is likely the cause of the positive result and the investigation should focus on post sterilization BI handling and processing.

ISO 11138-7 *Sterilization of health care products – Biological Indicators – Part 7: Guidance for the selection, use and interpretations of results*
*10.2 Interpretation of results*
*The identification of growth as that of the test organism should be confirmed ...*

USP General Chapters <1229.5> *Biological Indicators for Sterilization*
*Characterization of Biological Indicators – Identification*
*Where identification of the BI species is deemed necessary, as in the course of an investigation into unusual results, use either a phenotypic or genotypic identification method ...*

Mesa Labs recommends reading BI results frequently throughout the incubation period. This recommendation is twofold. First, it allows for a facility to respond as soon as possible to the event of a positive BI result. Second, it is imperative to begin the identification process as soon as a positive BI is noticed, otherwise it may be impossible to obtain growth of the vegetative cells needed to perform the identification.

BIs are test systems inoculated with spore forming bacteria. Spores are a dormant form of the bacteria and will remain in this dormant state until provided with a nutrient source, growth media, and the correct growth temperature during incubation. With these favorable conditions, the spores will germinate into metabolically active vegetative cells which begin to proliferate while metabolizing carbohydrates in the growth media. The result of the carbohydrate metabolism is an acidic by-product that lowers the pH of the growth media. If the media contains a pH indicator such as Bromocresol Purple or Phenol Red, the lowering of the pH due to the acidic by-product is what causes the visual appearance of the media to change from purple or red to yellow. The same mechanism is at work in media that does not contain a pH indicator, it is just not visually observable as a color change but rather as turbidity. The acidic by-product is lethal to the vegetative cells and if left in the unfavorable environment too long, all cells will die making it impossible to obtain growth for organism identification.

Below is a step by step procedure recommended for sub-culturing a positive Mesa BI. All operations should be performed in a clean bench or biosafety cabinet so as not to introduce contamination which may lead to incorrect identification of the organism that caused the positive result. Additionally, bacteriostatic, bactericidal, sporostatic or sporicidal agents should not be used to disinfect the external surfaces of a self-contained biological indicator (SCBI) prior to sub-culturing.

**Sub-culturing Procedure**

1. Access the growth media.

    - For EZTest® or ExpoSure®, carefully remove the cap and filter paper (EZTest) from the SCBI.

    - For BIs cultured in a test tube with growth media, aseptically open the test tube by removing the cap.

    - For liquid submersible BIs, ProSpore, MagnaAmp™ and SterilAmp®, disinfect the outside of the ampoule with Isopropyl Alcohol and allow to dry. Place ampoule in a sterile Pyrex bottle and crush using either a sterile stainless-steel rod or sterile forceps (safety goggles should be worn as a precaution).

2. Using a 1.0 mL pipette or micropipette with a long tip, extract some of the liquid from the SCBI unit, the test tube, or the Pyrex bottle. The glass fragments at the bottom of an SCBI might impede this process. Extract as much of the liquid as possible.

3. Dispense liquid onto a prepoured and solidified Trypticase Soy Agar (TSA) plate. Using accepted microbiological culturing techniques spread the liquid evenly across the agar.

4. Incubate the plate for at least 24 hours at 55-60°C for *Geobacillus stearothermophilus* or 30-35°C for *Bacillus atrophaeus*.

5. Perform appropriate biochemical tests (see table below) or genetic identification on the colonies from the TSA plates in order to confirm microorganism identity.

| **Test** | ***G. stearothermophilus*** | ***B. atrophaeus*** |
|---|---|---|
| Gram Stain | Gram (+) Rod | Gram (+) Rod |
| Methyl Red – Vogues Proskauer (MRVP) | Negative (-) | Positive (+) |
| Catalase | Negative (-) or Weak Positive (+) | Positive (+) |
| Acid production in presence of carbohydrate source | Positive (+) | Positive (+) |
| Growth at 65°C | Positive (+) | N/A |
| Dextrose Agar | N/A | Orange Pigmented Colonies |
| Tyrosine Agar | N/A | Brown/Black Pigmented Colonies* |
| Dextrose Starch Agar | N/A | Positive (+) (= starch hydrolysis) |

*5 to 7 days incubation required to observe black pigment

Written by Nicole Robichaud, Technical Trainer for Mesa Labs Sterilization and Disinfection Control Division. Nicole began working for Mesa Labs in 2007 as a Microbiological Scientist in the Spore Cultivation Laboratory and has held positions in Quality Control and Technical Support. Nicole holds a B.S. in Biological Sciences from Montana State University. She is a member of the Association for the Advancement of Medical Instrumentation (AAMI) and the Parenteral Drug Association (PDA).