

SAFE OPERATING PROCEDURE

AUTOCLAVE EFFICACY TESTING: BIOLOGICAL INDICATORS

Purpose:

This protocol outlines the operating procedure to monitor the efficacy of autoclaves in sterilizing biohazard waste with the use of self-contained biological indicators (SCBI). This procedure has been written for use with: VERIFY™ Dual Species Self-Contained Biological Indicators by STERIS.

Biological Indicators:

Biological indicator testing is used to determine the efficacy of the autoclave process and determine that proper sterilization of the contents has occurred. Each indicator is completely self-contained, combining a disc inoculated with dual spore species (*Geobacillus stearothermophilus* and *Bacillus atropaeus*) and an ampule of specifically formulated soybean casein digest growth medium with a pH indicator. Chemical indicators on each vial provide immediate proof of processing. Following incubation, a vivid color change from deep blue/purple to bright yellow and/or turbidity gives unmistakable evidence of microbial growth. If no microbial growth occurs, the vial remains deep blue and without turbidity.

G. stearothermophilus spores are commonly used as a challenge organism for sterilization validation studies and periodic checks of sterilization cycles as they are the most resistant organism to steam sterilization. To determine the effectiveness of the autoclave process the biological indicator must be placed in a typical test load (solid or liquid) and exposed to the typical cycle conditions.

Safety Precautions:

- ⚠ Refer to the Standard Operating Procedure for autoclave operation for details on the potential hazards associated with autoclave use, operator instructions and contingency plans for spills, emergencies, and malfunctions.
- ⚠ It is important that you do not handle the biological indicator until the ampoule has cooled. Excessive handling may result in personal injury caused by flying debris.
- ⚠ Appropriate safety equipment (gloves, eye protection, and lab coat) must be used when removing biological indicators from the sterilizer.

Biological Indicators are to be run bi-weekly on all 4 user sterilizers in the Department of Molecular and Cellular Biology. **Sterilizer locations: SCIE: 4402B/4223B/3402A/3222B.**

1. Label the Steris Verify® SCBI vial with the date, and sterilizer room number.
2. Place the single Steris Verify® SCBI vial into in a small beaker (autoclavable), place a piece of autoclave tape across the top of the beaker.
3. Place in the middle of a fully loaded autoclave bin. This serves as the area most challenging for the sterilizer to penetrate.
4. Load the sterilizer as normal and run autoclave cycle “Liquid 30”.
5. **CAUTION:** After sterilization has been completed (and aeration, if required), allow the Verify Self Contained Biological Indicator (SCBI) to cool until safe to handle. Observe the chemical process indicator on each vial label to verify the color change corresponding to the sterilization cycle; i.e. steam turns the steam process exposure indicator to black. If the chemical indicator is unchanged, exposure to the sterilization process may not have occurred.

The SCBI vial must be ‘activated’ before it is incubated. To activate the indicator vial:

6. insert the vial into the Verify® Activator (black cylinder)
7. Place the blue cap over both the vial and the activator and press down firmly. The Verify SCBI is properly sealed when the cap is pushed down to the second black bar on the vial label.
8. Pull the vial outward through the opening on the side of the activator (Note: there is some resistance). This compresses the sides of the vial and ensures contact between the growth medium and the spore disc. Verify the spore disc is submerged in the media by gently tapping the SCBI bottom on a hard surface.
9. Activate a second indicator vial **from the same lot** that has not been autoclaved to serve as a positive control.
10. Place the activated indicator vials and the activated control vial in a heating block. The incubation temperature is set to 55-60°C (131-140°F) following a steam sterilization cycle.
11. After 24 hours incubation, examine the biological indicator vials for color change.
12. If the media in the test SCBI begins to show turbidity and/or color change from deep blue to yellow, sterility has not been achieved. If sterilization of the indicator was achieved, the SCBI shows no change during the incubation period; i.e., there is no turbidity and the growth media remains deep blue.
13. If the autoclaved test indicator vial has visible turbidity and/or a visible colour change from blue to yellow at 24 hours, steam sterilization has not occurred. (The media within the unsterilized positive control vial should change from blue to yellow, and have visible turbidity whilst the media in the autoclaved test indicator vial should remain blue).
14. In the event of a positive result with a test vial, re-test the sterilizer with an additional SCBI. If it displays another positive result, place the autoclave out of service and contact CBS Health & Safety Officer.
15. Record the date of the test, test results and all other pertinent information in the log book located in the drawer of each autoclave room.

Storage Conditions:

- Verify™ SCBI's should be stored at a controlled room temperature (20-25°C) and 30-60% R.H. Do not store in close proximity to steam or ethylene oxide (EO) processing areas. Avoid contact with, or storage near, sterilants or chemicals; i.e., any oxidizing or reducing agents such as formaldehyde, bleach, ammonia, etc.
- Do not use after the expiration date printed on the packaging.

Disposal:

- Before discarding, treat as appropriate for standard microbiological waste, non-pathogenic species. Expired vials should be autoclaved before disposal. Autoclave: used, expired and positive controls at 121°C/250°F for 30 minutes.

Contingency Plans:

- VERIFY™ Biological Indicators are NOT hazardous products according to the OSHA Hazard Communication Standard, 29 CFR 1910.1200.

In the case of accidental exposure, take the following precautionary measures:

- EYES: Immediately flush eyes with plenty of water for 15 minutes. Get medical attention if irritation persists.
- SKIN: Immediately wash with soap and water. Get medical attention if irritation develops.
- INGESTION: Give water to drink and get medical advice.
- INHALATION: Remove to fresh air. Get medical attention for any breathing difficulty.