

EH&S INTERNAL

STANDARD OPERATING PROCEDURE

Performance Verification of Steam Autoclave Kill Cycle with Heat Sink Concept

BACKGROUND

Biological waste is an important occupational hazard for people who work with the waste products of research and teaching laboratories. Biological (or special) waste has been defined in [Title 30 Texas Administrative Code \(30 TAC\), Chapter 330, Section \(§\) 330.3\(148\)](#) as waste which requires special handling to protect human health or the environment. Biological waste is regulated by [the Texas Commission on Environmental Quality \(TCEQ\)](#) and the [Texas Department of State Health Services \(TDSHS\)](#). It is very important to be able to assure that viable biohazardous organisms are not sent to the landfill. If adequate steam does not have adequate contact with biohazardous materials, microorganisms can survive a trip through the autoclave. Autoclave tape is not a reliable means to determine if the time, temperature, and pressure combination of the process was adequate to penetrate and kill microorganisms contained within the load.

BIOLOGICAL INDICATORS

UT Arlington (UTA) autoclaves used for kill loads are tested semiannually by Environmental Health and Safety Office (EH&S) for killing effectiveness through the use of **biological indicators**. Spore ampoules are self-contained biological indicators and they are intended for use in the monitoring of saturated steam sterilization cycles at 121°C (250°F). Commercially available *Geobacillus stearothermophilus* spore ampoules (ProSpore ampoules, picture 1) have average spore populations of 10⁵ organisms. ProSpore comply with [AAMI/ISO 11138-1](#) and [AAMI/ISO 11138-3](#). Spores are suspended in growth medium containing bromocresol purple to function as a pH indicator. The acid production associated with growth causes a change in color from purple to or toward yellow.

Spore ampoules:

- should be stored in a refrigerator at 2-8°C
- should not be frozen
- should not be used if damaged
- should not be used after expiration date
- should be handled with care since they contain live cultures
- are not intended for flash sterilization processes

STEAM CHEMICAL INTEGRATORS

3M Comply™ Thermalog™ Steam Chemical Integrators ([Steam Chemical Integrator](#), picture 2) are also used by EH&S simultaneously with biological indicators for testing autoclave killing effectiveness.

AUTOCLAVE WASTE DECONTAMINATION CYCLE TESTING & VERIFICATION & PERFORMANCE CHECKS

In addition to the above-mentioned autoclave semiannual testing, EH&S also test autoclaves when repairs have been performed on them. If test results indicate that the autoclave is not sterilizing properly, the autoclave should not be used for waste until it has been serviced again. A notice shall be placed on the autoclave indicating that it is not to be used until the problem is diagnosed and corrected. The first load run in the autoclave after repair should be the test run with biological indicator and also including a Steam Chemical Integrator in the run to ensure proper functioning of the autoclave.

Preparing the Heat Sink Concept

The Heat Sink Concept was developed by UTA Life Sciences Core Facility Director Dr. Kimberly Bowles during summer months of 2016.

Heat Sink:

- Autoclavable secondary container (picture 3)
- In the container an autoclavable plastic bag (picture 3)
- In the autoclavable plastic bag three to four glass bottles (borosilicate - Kimax or Pyrex), each containing water (total amount 1600 ml). Caps on the bottles are kept loose.
- Steam Chemical Integrator is attached with autoclave tape outside a small polypropylene container (picture 4)
- ProSpore ampoule is marked with autoclave tape above the neck of the ampoule with the name of the autoclave to be tested and secured inside the polypropylene container horizontally with autoclave tape (pictures 5 and 6, picture 7)
- The autoclavable plastic bag is left slightly open to allow for steam penetration (pictures 8 and 9)

Autoclaving

- Autoclave the Heat Sink Concept selecting the time and temperature as 50–65 minutes at 121°C (250°F) according to the cycle indicated for liquid waste for the particular autoclave to be tested
- Allow time for the autoclave to cool down and for pressure to return to atmospheric after the cycle is finished.
- Once the cycle is complete, remove Heat Sink Concept from autoclave using insulated gloves or heat resistant mitts, long sleeve lab coat / rubber apron, and safety glasses, and let contents cool.
- Inspect the [Steam Chemical Integrators](#) and interpret the results.
- After sterilization, handle ampoules with care. Contents of the ampoules are hot and under pressure.

Incubation, monitoring and result interpretation of the biological indicator ampoule

- Place the processed biological indicator ampoule in a glass test tube (vertical position) in a water bath (pictures 10 and 11). Water temperature needs to be 55-60°C.
- Mark a control indicator ampoule as such and incubate along with processed ampoule to ensure spore viability. Incubate totally for 48 hours.
- Examine the biological indicator ampoules daily during incubation. Check for signs of growth at regular

intervals during the incubation period (24 and 48 hours). Record observations. All positive ampoules should be recorded and then disposed of by incineration.

- Interpretate the results (picture 12):
 - **Control:** The control ampoule should exhibit a color change to or toward yellow and/or show turbidity. If the control ampoule shows no signs of growth, consider the test invalid.
 - **Test:** A failed sterilization cycle is indicated by turbidity and/or a change in color to or toward yellow. A test ampoule that retains its purple color indicates an adequate sterilization cycle.

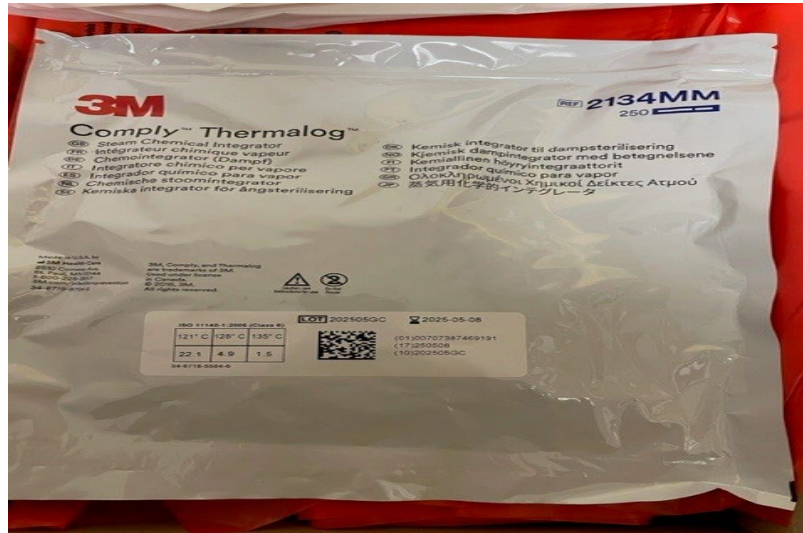
Record Keeping

A log of each test should be maintained, which includes the type of indicator used, test date, and result of the test. The [Biological Indicator Test Results Log](#) (CO-EHS-F502) is available for download.

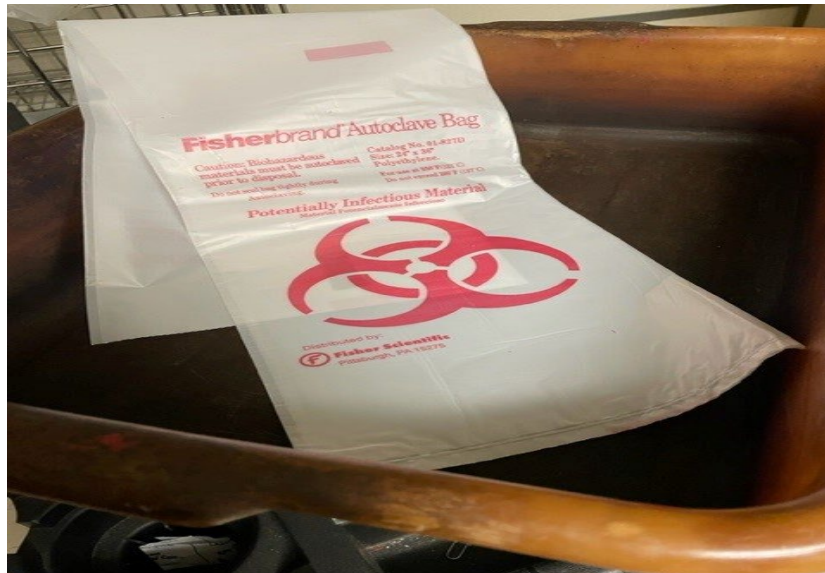
Picture 1. ProSpore Ampoules



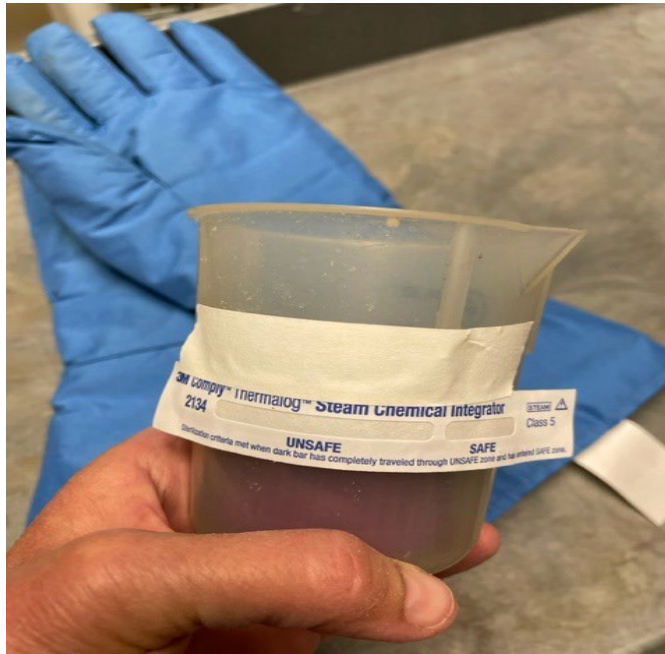
Picture 2. 3M Comply™ Thermalog™ Steam Chemical Integrators



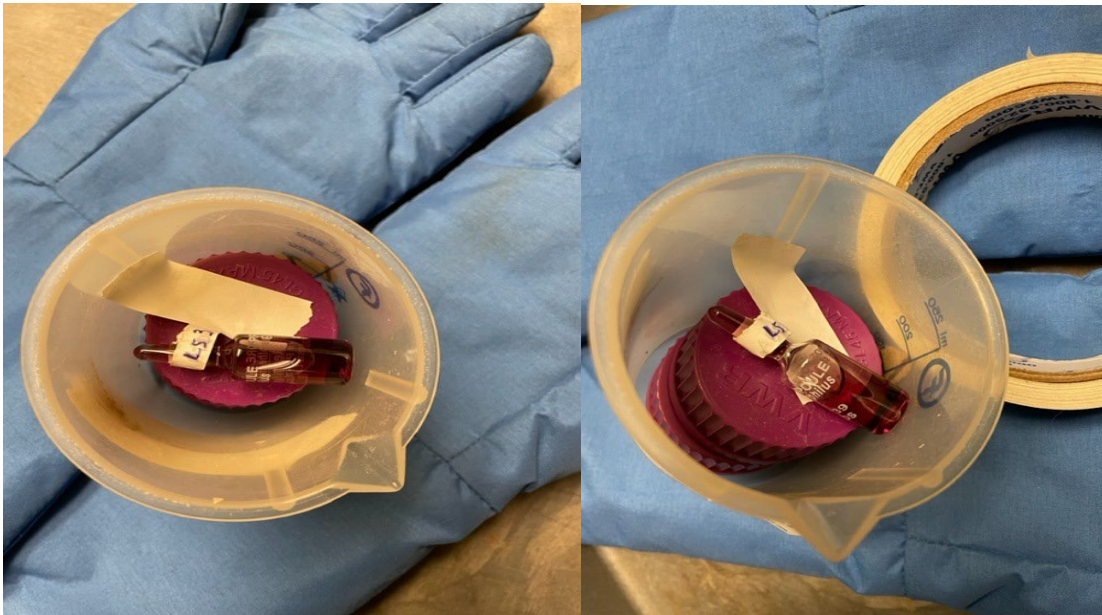
Picture 3. Autoclavable secondary container and autoclavable plastic bag



Picture 4. Steam Chemical Integrator attached with autoclave tape outside a polypropylene container



Pictures 5 & 6. ProSpore ampoule is secured inside the polypropylene container horizontally with autoclave tape



Picture 7. Location of both the Steam Chemical Integrator and the ProSpore ampoule



Pictures 8 & 9. The Heat Sink Concept is ready. The autoclavable plastic bag is closed with autoclave tape but left slightly open

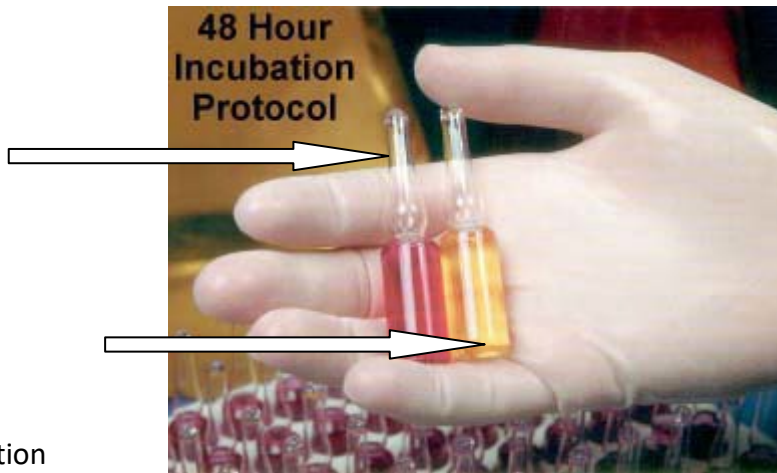


Picture 10 & 11. Biological indicator ampoules in glass test tubes (vertical position) in a water bath



Picture 12. Interpretate the results of the biological indicator ampoule

A test ampoule retaining its purple color indicates an adequate sterilization cycle



A failed sterilization cycle is indicated by turbidity and/or a change in color to or toward yellow