ENVIRONMENTAL HEALTH AND SAFETY
University of Nebraska-Lincoln

Safe Operating Procedure

(Revised 5/25)

AUTOCLAVE PERFORMANCE TESTING FOR BIOHAZARDOUS WASTE

Introduction

Autoclaves that are used to sterilize biohazardous waste need to be regularly serviced, maintained, and tested for performance. Testing confirms proper autoclave function and reduces the risk of unintentional release of biohazardous waste materials into the environment due to a malfunctioning autoclave. A primary method of performance testing of autoclaves by users is the use of a biological indicator (BI). Biological indicator (BI) ampoules/vials contain 10^6 population of *Geobacillus stearothermophilus* spores and are designed to demonstrate whether the conditions during a steam autoclave cycle were able to achieve a defined level of microbial inactivation. These biological spores can be purchased from many scientific supply vendors. Common manufacturers include 3M Attest, Apex, Mesa, STERIS and McKesson.

> NOTE: Autoclave tape is NOT a biological indicator. The autoclave tape **only** indicates that the tape was exposed to steam and hot temperatures in the autoclave chamber. It **does not** reflect time of steam exposure or temperature/ conditions inside the biohazardous waste bag.

Recommended Performance Testing Frequencies (with BI)

All materials containing pathogens (animal, arthropod, plant, human), recombinant or synthetic nucleic acid molecules (r/sNA), biological toxins, or other regulated material **must be** autoclaved prior to disposal.

Recommended autoclave performance testing frequency is based on the containment level of the agents in use. Consult the EHS SOP **Biosafety Containment Levels** for a detailed description.

- **Quarterly:** minimum recommended testing frequency for laboratories working with material in **BSL-1, ABSL-1, BL1-P containment**. (For example, plant pathogens, as well as human Risk Group 1 agents, are typically at this containment).
- **Monthly:** minimum recommended testing frequency for laboratories working with material in **at BSL-2, ABSL-2, BL2-P and large scale BL1-LS containment**. (for

example, work with human, animal (including arthropods) and plant pathogens, toxins, human and non-human primate materials are typically at this containment).

- **Per-load: BSL-3 and ABSL3** laboratories and labs working with Risk Group 2 or Risk Group 3 organisms or select agents and/or select toxins are typically at this containment.

BI Testing Procedure

When conducting performance testing with BI's, and not autoclaving an actual biohazardous waste load, it is important to simulate a typical autoclave load when placing the BI (tubes, plates, pipets, towels, absorbent liners, gloves, necropsy materials, animal caging…). This can be done by placing the BI in a location that is not easily reached by steam, like the middle of an autoclave bag. The procedures below were developed to achieve this environment for different autoclave types.

1. Inspect the BI for damage or cracks, verify expiration date, and familiarize yourself with the manufacturer instructions for use and subsequent incubation.
2. To perform the test, follow the procedures below with the cycle used for decontaminating biohazardous waste (See EHS Biosafety See EHS SOP, ***Autoclave Operation and Use***):

   2.1 **For dry loads (Gravity or Pre-vacuum cycle)** obtain a pipet tip storage box with the pipet tip insert still in the box (Figure 1A) and (1) place the BI on its side inside the box on top of the pipet tip insert. (2) Use autoclave tape to secure the BI. (3) Close the lid of the box and place the box under your biohazardous waste load (i.e., under the autoclave waste bag(s) Figure 1B).

   - You can also place the BI on a string/ piece of yarn and place it into a mockup "biohazard waste" load as shown in Figure 2A and 2B.

   2.2 **For liquid biological waste loads (Liquid cycle)**, using a piece of string tied around the BI, suspend the BI in the liquid and secure the string to the outside of the container with autoclave tape (Figure 1C). **IMPORTANT: ONLY USE glass ampoule-style BIs** for testing liquid loads and make sure the cap on the liquid bottle/ container is loose.

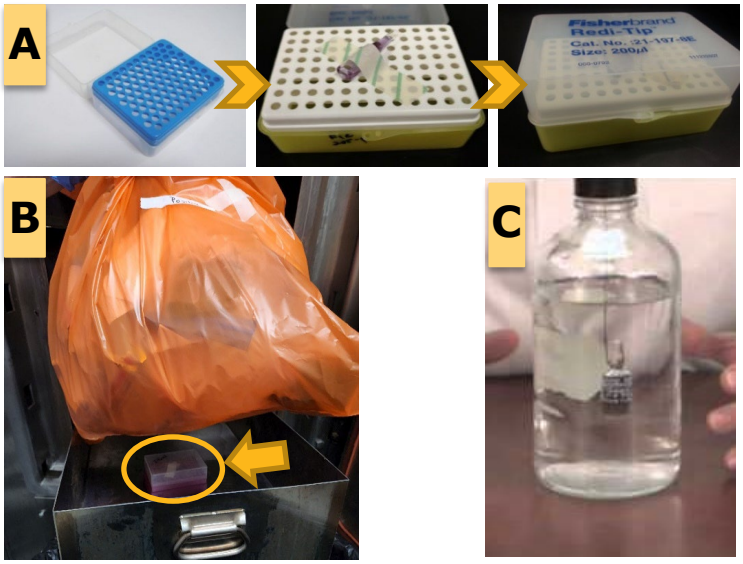

Figure 1 above shows Biological Indicator prep and placement for standard autoclaves. **A**. preparation of BI in pipette tip box; **B**. placement of tip box under load in bottom of pan (yellow circle); **C**. hanging BI from string inside liquid container with loose cap.

Figure 2A

Figure 2B

2.3 **For tabletop and top-loading portable autoclave/sterilizers**, (Figure 2 above). Obtain a 50 mL conical centrifuge tube. (2) Place the BI in the tube. (3) Place one piece of autoclave tape over the mouth of the tube to prevent the BI falling out. It is important to allow steam to enter the tube.

Figure 2A shows attaching string to a biological indicator in preparation for Figure 2B. Using another piece of autoclave tape, secure the tube to the bottom of the autoclave bag or simply place the tube under the biohazard bag(s) in the bottom of the basket (Figure 2B).

3. **For plant material and soil in bags** use either method 2.1 or 2.3 outlined above or as shown in Figures 3 following.

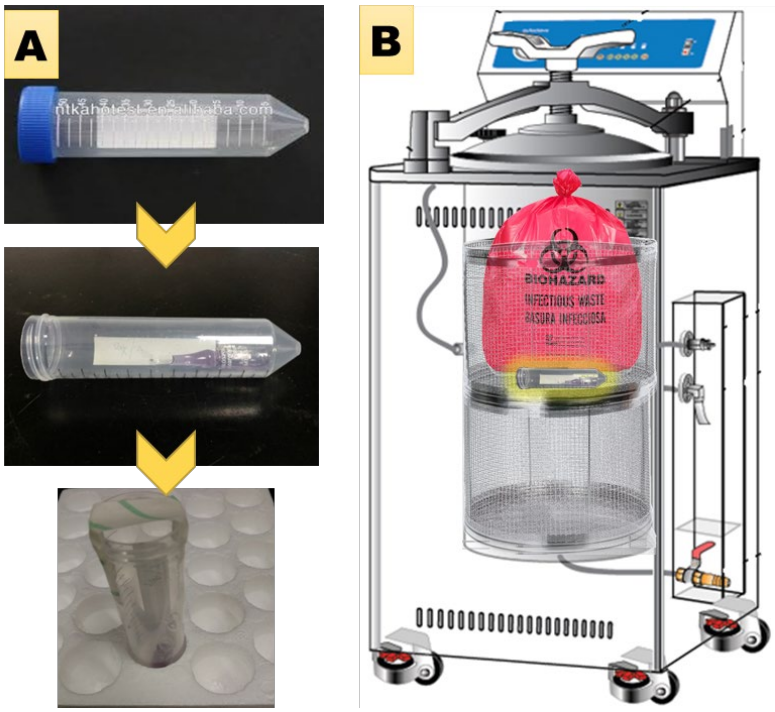

Figure 3 Biological Indicator prep for top-loading/tabletop autoclaves A. prep of BI in 50mL conical tube; **B**. Placement of conical tube (yellow highlight) under load in bottom of the basket. The lid should be loosely placed back on the centrifuge tube (Do not seal the BI in the centrifuge tube)

4. Autoclave using appropriate cycle/settings. For difficult loads, increase sterilization time and/ or temperature. See EHS SOP, **Autoclave Operation and Use** for guidance on cycle development or contact EHS at 402.472.4925.

Minimum small biohazard waste cycle settings are 121°C, 15-17 psi with a sterilization phase of at least 60 minutes. 130°C, 17-28psi with a sterilization phase for 60 minutes may be needed for larger biological waste loads.

5. Allow time for the autoclave to cool down and for pressure to return to atmospheric.
6. Using proper PPE (eye protection, lab coat, insulated gloves or mitts, close toed shoes…), remove load from autoclave.
7. Remove BI pipet tip box from bottom of tray and open box to remove BI, pull the BI out of the load using the yarn/ string, or remove cover from liquid container and retrieve BI from liquid.

Incubation of Test BIs

When the autoclave is complete, the biological indicator is removed from the autoclave, disinfected as applicable, and brought to the incubator room.

Note: The BI is hot and under pressure after autoclaving so make sure to let it cool before attempting to touch it.

indicators must be incubated at a specified temperature (typically 57-59°C) for 24-48 hours. **See BI manufacturer's instructions**. It is the presence or absence of growth that indicates the proper performance of the autoclave cycle. Some indicators require release of the growth medium from a glass ampoule inside the BI plastic cover prior to starting the incubation process (e.g., 3M Attest, STERIS, and McKesson biological indicators).

After the proper incubation period (see manufacturer's instructions), the BI can be examined for signs of growth. The BIs contain a colorimetric compound that causes a color change from purple to yellow in the presence of bacterial growth (Figures 4A and 4B). The solution may also become cloudy, indicating growth. You will also want to run a control using a BI that has NOT gone through an autoclave run. The control should be yellow after incubation, whereas the BI that went through the autoclave cycle should remain purple. Make sure to order the appropriate BI for the temperature of the cycle you will be using.

Figure 4A above shows vials with color change from purple to yellow in the presence of bacterial growth.

Vial from box No color change Growth/Color change

Figure 4B above shows a sample tube before testing (left image), tubes with no color change indication for spore growth (middle image). No growth is indicated by purple color. Right image shows growth is indicated by color change to yellow and increased turbidity (cloudiness).

Recording Results

Keeping a record of the results is the responsibility of the lab or group of labs performing the test. EHS only maintains historical testing records completed prior to December 31, 2022, except for autoclaves in BSL-3 laboratories on campus and autoclaves used for destruction of Biological Select Agents and Toxins. Certain regulatory agencies (e.g., USDA-APHIS) require proof of the proper function of an autoclave used to destroy regulated material. A sample Autoclave Log Sheet (Figure 4) can be found on the EHS website at: https://ehs.unl.edu/sites/unl.edu.business-and-finance.university-operations.ehs/files/media/file/autoclave_log_sheet.pdf

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AUTOCLAVE LOG SHEET

Loads containing biohazardous waste must be autoclaved at ≥ 121°C with a sterlization time of at least 30 minutes.

Autoclave make/model:		**Location (Building):**	
Person Responsible for autoclave:		**Location (Room Number):**	
Contact Phone Number:		**EHS Autoclave ID:**	

Date	Load Contents	Cycle number	Cycle Type	Sterilization Time (min)	Pressure (psi)	Max Temp Reached	Tape Result (pass/fail)	Chemical Indicator* Result (pass/fail)	Biological Indicator Used? (Y/N)	Operator	Comments

*Chemical indicator = autoclave tape or chemical integrator pack.

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Figure 2 Sample Autoclave Log Sheet

Autoclave Testing Following Malfunction or Repair

Follow these instructions if the autoclave utilized for decontamination of biohazardous waste aborts or there is a malfunction during operation:

1. Post an “out of order” or “NOT IN SERVICE” sign. The sign should include contact information for the person coordinating repairs and list an alternate autoclave to use, if one is available, while repairs are completed.
2. Notify your supervisor and the autoclave custodian in charge of testing the autoclave.
3. Contact the service provider for the autoclave to complete the repair.
4. Verify and document proper performance following repair:
   a) Get documentation that the service provider performed testing or
   b) Run a load with a BI following the procedure in this document.
5. Remove the posted “out of order” sign and resume normal operation.