ENVIRONMENTAL HEALTH AND SAFETY
University of Nebraska-Lincoln

Safe Operating Procedure

(Revised 5/23)

AUTOCLAVE PERFORMANCE TESTING

Introduction

Autoclaves that are used to sterilize biohazardous waste should be regularly serviced, maintained, and tested for performance. Testing confirms proper autoclave function and reduces the risk of unintentional release of biohazardous waste materials due to a malfunctioning autoclave. A primary method of performance testing of autoclaves by users is the use of a biological indicator (BI) because autoclave indicator tape **does not** prove decontamination effectiveness. Autoclave tape only indicates that the tape was exposed to temperatures commonly used in sterilization processes, typically 121°C, it does not accurately reflect time of exposure or conditions inside the load.

Biological indicator (BI) ampoules/vials (containing 10^6 population of *Geobacillus stearothermophilus* spores) can be purchased from many scientific supply vendors. Common manufacturers include 3M Attest, Apex, Mesa, and McKesson.

Assessment of the risk of the biohazardous waste being decontaminated in the autoclave (i.e. laboratory containment level and biological materials in use) should be used to determine a recommended testing frequency.

Recommended Performance Testing Frequencies (with BI)

All materials containing recombinant or synthetic nucleic acid molecules (r/sNA) pathogens (animal, plant, human), 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 at BSL-1, ABSL-1, BL1-P containment. (For example, animal and plant pathogens, as well as human Risk Group 1 agents are typically at this containment).
- **Monthly:** work with human pathogens, toxins and some human and non-human primate materials as well as some animal and plant pathogens that are used at BSL-2, ABSL-2, BL2-P and large scale BL1-LS containment.
- **Per-load:** BSL-3 laboratories and labs working with select agents and/or select toxins.

BI Testing Procedure

When conducting performance testing with BI's and not autoclaving an actual waste load, it is important to simulate a typical load when placing the BI in the chamber. This can be done by placing the BI in a location that is not easily reached by steam, similar to 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 manufacturer instructions for use and subsequent incubation.
2. To perform the test, follow the procedures below with the cycle used for decontaminating waste:

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

   2.2 **For liquid loads**, 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.

   2.3 **For tabletop and top-loading portable autoclave/sterilizers**, (Figure 2A) (1) 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

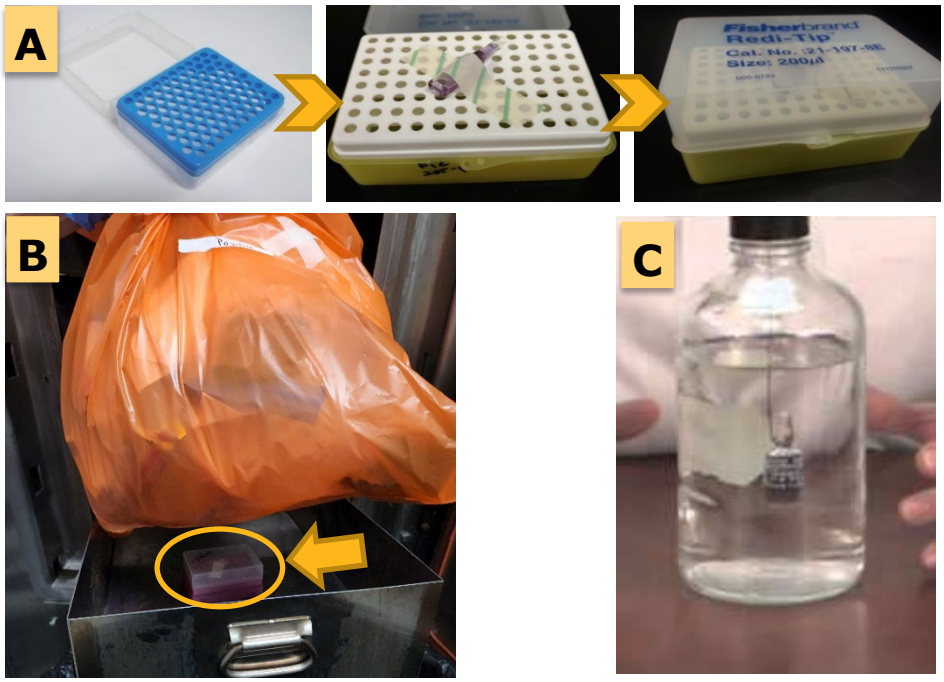

Figure 1 Biological Indicator prep and placement for standard autoclaves. ***A****. preparation of BI in pipette tip box;* ***B****. placement of tip box under load in bottom of pan (yellow circle);* ***C****. hanging BI from string inside liquid container with loose cap.*

important to allow for steam to enter the tube. **DO NOT PLACE THE LID BACK ON THE TUBE**

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).

2.4 **For plant material and soil in bags,** use either method 2.1 or 2.3 outlined above and shown in Figures 1 and 2.

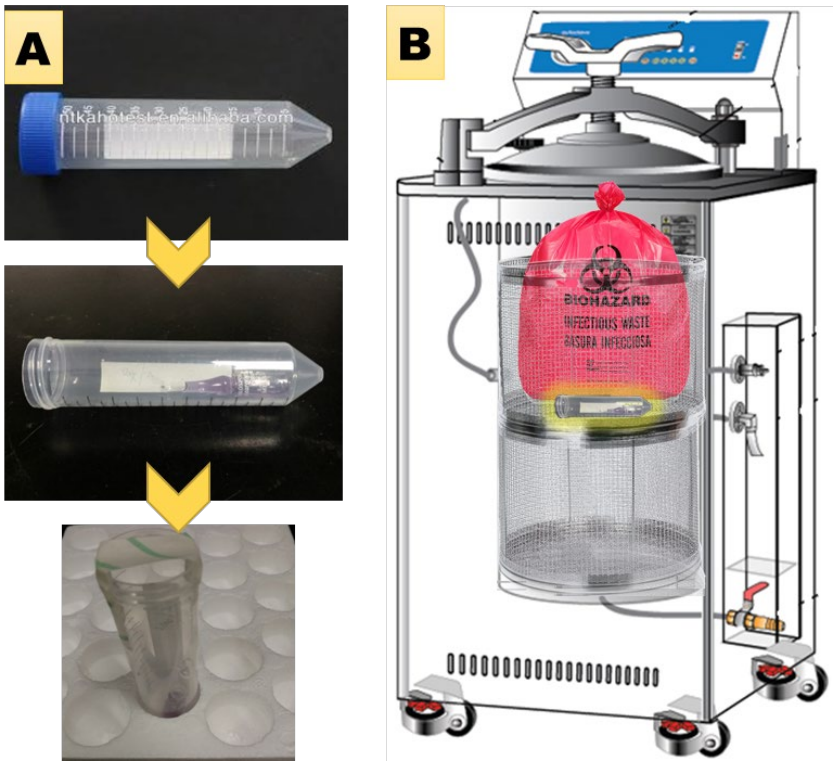

Figure 2 Biological Indicator prep for top-loading/tabletop autoclaves *__A__. prep of BI in 50mL conical tube; __B__. Placement of conical tube (yellow highlight) under load in bottom of the basket.*

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

Minimum waste cycle settings are 121°C, 15-17 psi with a sterilization phase of 30 minutes.

4. Allow time for the autoclave to cool down and for pressure to return to atmospheric.
5. Using insulated gloves or mitts, remove load from autoclave.
6. Remove pipet tip box from bottom of tray and open box to remove BI or remove cover from liquid container and retrieve BI from liquid.

Incubation of test BIs

Biological indicators must be incubated at a specified temperature to support bacterial growth. 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 prior to starting the incubation process (e.g., 3M Attest indicators). Typically incubation of the BI at 57-59°C for at least 48 hours is required. After 48 hours, 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 (Figure 3). The solution may also become cloudy indicating growth.

Figure 3 *Color change indication for spore growth. No growth is indicated by purple color (left), growth is indicated by color change to yellow and increased turbidity (cloudiness) (right).*

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 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/autoclave_log_sheet.pdf

Figure 4 Sample Autoclave Log Sheet

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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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Autoclave Testing Following Malfunction or Repair

For autoclaves utilized for decontamination of biohazardous waste, if a run aborts or there is a malfunction during operation, please follow these instructions:

1. Post an out of order 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 a repair.
4. Verify performance following repair:
   a) Service provider performed testing; or
   b) Run a load with a BI following the procedure in this document.
5. Remove the sign and resume normal operation if performance is verified.
6. Repeat the autoclave cycle for any waste materials that were in a failed run.