

## AUTOCLAVE PERFORMANCE TESTING

### Introduction

As part of a campus-wide surveillance program, most autoclaves that are used to sterilize biohazardous waste are enrolled in the EHS “Autoclave Surveillance Program.” EHS created this program to assist researchers in confirming proper autoclave function to avoid unintentional release of biohazardous waste materials due to a malfunctioning autoclave. The surveillance program involves performance testing of autoclaves with a biological indicator (BI) because autoclave indicator tape **does not** prove decontamination effectiveness. Indicator tape only indicates that the outside of the container came to temperature, it does not reflect time of exposure or conditions inside the load.

Autoclaves enrolled in this program are tagged with a sticker (see below). Orange labels are placed on autoclaves tested monthly or on a per-load basis and white labels are placed on autoclaves tested quarterly (every 3 months). Each autoclave is assigned an identification number based on the room where it is located and the number of autoclaves in the room.

| UNL EHS Autoclave Testing Program   |                        |
|---|------------------------|
| Building:   | <b>Morrison Center</b> |
| ID#:  | <b>119-1</b>           |
| <i>If this unit is moved or replaced, please contact EHS at 402.472.4925.</i> |                        |

| UNL EHS Autoclave Testing Program   |                  |
|---|------------------|
| Building:   | <b>Keim Hall</b> |
| ID#:  | <b>329D-1</b>    |
| <i>If this unit is moved or replaced, please contact EHS at 402.472.4925.</i> |                  |

***If you need to contact EHS about an autoclave, please reference the Autoclave ID# listed on the sticker.***

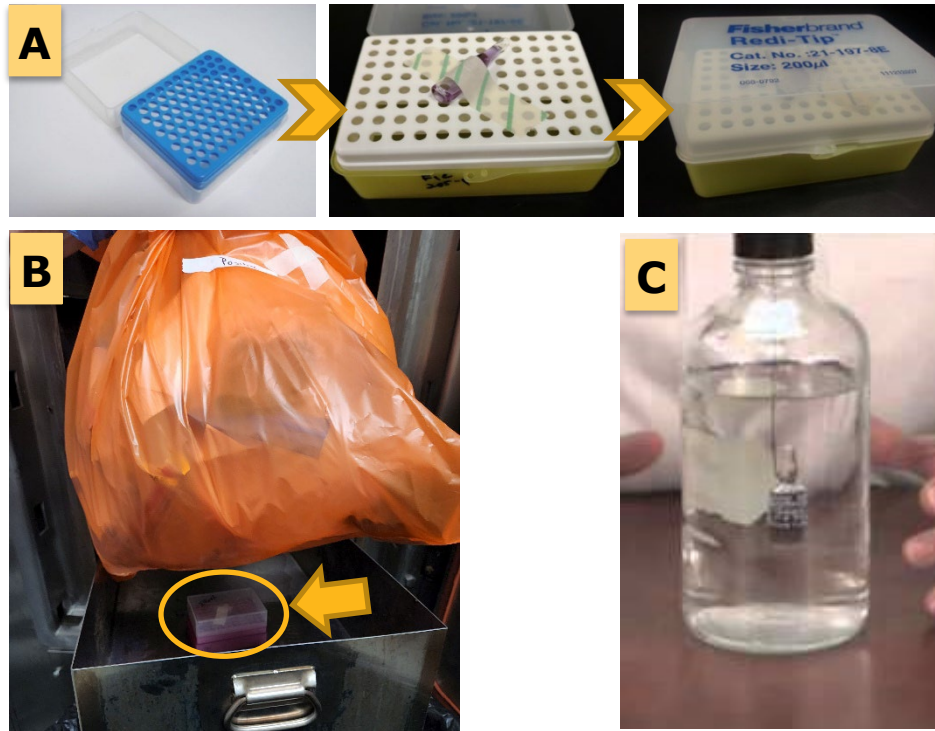
EHS distributes performance test kits that contain the following: a biological indicator (BI) ampoule (contains 10<sup>6</sup> population of *Geobacillus stearothermophilus* spores), shipping tube, documentation form, and instructions. These kits are sent out to autoclave “operators” at regular intervals according to the assigned performance surveillance frequency of the autoclave(s). The assigned surveillance frequency is assigned based on a risk assessment of the biohazardous waste routinely decontaminated in the autoclave.

## Surveillance Frequencies

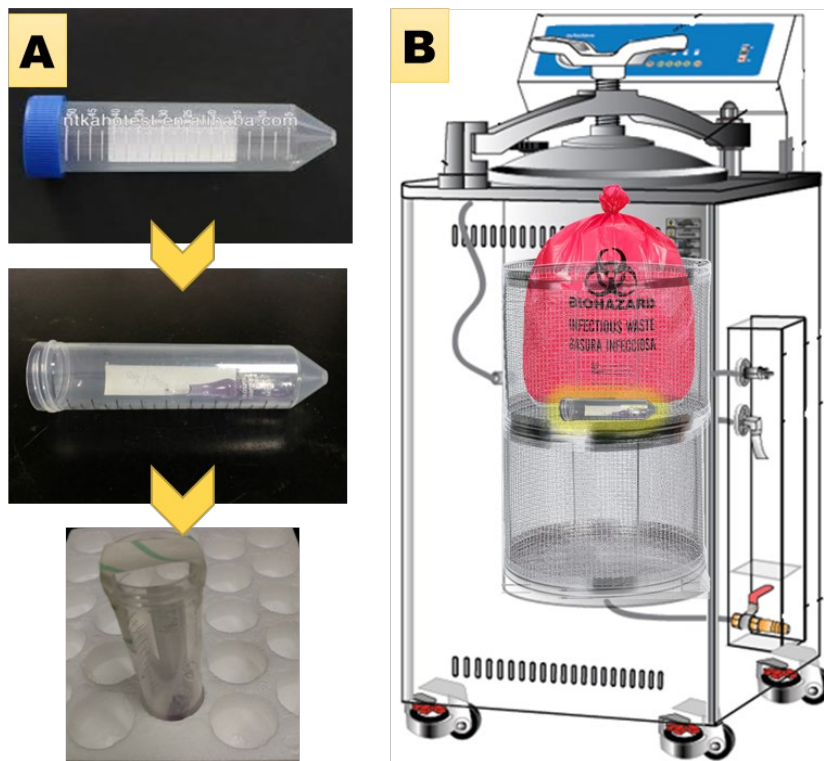
- **Monthly:** work with human pathogens, toxins and some human and non-human primate materials
- **Quarterly:** not working with human pathogens, toxins or certain human or non-human primate materials.
- **Per-load:** BSL-3 laboratories and labs working with select agents and/or select toxins.

## Testing Procedure

1. Open envelope and remove Falcon (50mL conical) tube and documentation form. If the packing material appears wet and the ampoule is cracked, contact EHS (402.472.4925) for a replacement ampoule.
2. Open falcon tube and remove packing material.
3. Remove ampoule and inspect for cracks. If you will be autoclaving the same day as receipt, hold the ampoule at room temperature until use. If you will be autoclaving a different day, store the ampoule in a refrigerator at 4°C (40°F).
4. To perform the test, follow the procedures below with the cycle used for decontaminating waste:
  - 4.1 **For dry loads (gravity or vacuum cycle)**, obtain a pipet tip storage box (Figure 1A) and (1) place the ampoule on its side inside the box. (2) Use autoclave tape to secure the ampoule. (3) Close the lid of the box and place the box under your load (i.e., under the autoclave waste bag(s) Figure 1B).
  - 4.2 **For liquid loads**, using a piece of string tied around the ampoule, suspend the ampoule in the liquid and secure the string to the outside of the container with autoclave tape (Figure 1C).
  - 4.3 **For tabletop and top-loading portable autoclave/sterilizers**, (Figure 2A) (1) Obtain a 50 mL conical centrifuge tube. (2) Place the ampoule in the tube. (3) Place one piece of autoclave tape over the mouth of the tube to prevent the ampoule falling out. It is 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).
  - 4.4 **For plant material and soil in bags**, use either method 4.1 or 4.3 outlined above and shown in Figures 1 and 2.



**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.



**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.

- 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.**

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

## 9. BI Incubation test for growth

### a. User incubation of test ampoules. PREFERRED METHOD

- Take a picture of the ampoule prior to incubation.
- Incubate the ampoule at 57-59°C for at least 48 hours. After 48 hours, the ampoule can be examined for signs of growth. The ampoules 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.
- Take a picture of the ampoule after incubation.
- Complete the online **Autoclave Performance Check Log** (see below for link) and upload the before and after pictures of the ampoule. Paper logs will also be accepted and provided in the package with the ampoules.



**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).

- b. **EHS Incubation of test ampoules:** If you are sending the ampoule back to EHS for incubation, allow ampoule to cool before placing it in the Falcon (50mL conical) tube provided by EHS and secure with packing material. The following items *must* be in the envelope returned to EHS:

**ITEMS TO BE SENT BACK TO EHS WITH TESTED AMPOULE**

- Copy of printout(s) from autoclave.**
- Falcon tube with tested ampoule in packing material.**
- If using paper log, include the completed log.**

**Note:** *If assigned to test multiple autoclaves, but you are unable to perform multiple tests on the same day, store ampoule(s) in a refrigerator and return all ampoules when all tests have been completed.*

- Place the return address label on envelope.
- Place in campus mail or hand-deliver to EHS office. The envelope **SHOULD BE RETURNED** to EHS promptly after the run is completed.
- Complete the paper performance check log provided with the ampoule or the online **Autoclave Performance Check Log** form at this link or by scanning the QR code to the right:  
<https://forms.office.com/r/gSQuKLF9zq>



## Testing Results

Following submission of the Autoclave Performance Check Log, if the incubation was performed by the user, EHS will evaluate the result and make a final determination of PASS or FAIL. EHS will contact the user only if the results indicated a failure or if the result was interpreted incorrectly and a user submitted PASS result is in fact a failed test showing growth. If EHS will be incubating the test ampoules and checking for growth, the user will be contacted only if the ampoule shows signs of growth, in this case EHS will contact the user to arrange for a second confirmatory test.

If the second test also fails, EHS will instruct the user to place an “**Out of Order, Do Not Use!**” sign clearly on the autoclave and submit a repair order. An alternate autoclave to use until repairs are made must be indicated on the sign. A sample sign is included at the end of this document. Print the sign, fill in the blanks, and post on the autoclave in need of repair.



## **Autoclave Testing Following Malfunction or Repair**

For autoclaves enrolled in the EHS surveillance program, if a run aborts or there is a malfunction during operation, please follow these instructions:

1. Post an out of order sign (a sample sign is at the end of this document).
2. Notify the autoclave custodian in charge of testing the autoclave and notify EHS.
3. Contact the service provider for the autoclave to complete a repair. After repair is complete, contact EHS to receive a test ampoule to verify autoclave performance.
4. Run a load with the test ampoule following the procedure in this document.
5. Send the test kit back to EHS or incubate the ampoule as described above.
  - a. EHS will notify you of the test results and if no growth is observed in the ampoule the out of order sign may be removed and normal operation resumed.
  - b. For in lab incubation, please wait for confirmation from EHS of the test result before removing the sign and resuming normal operation.

# AUTOCLAVE OUT OF ORDER



## Do NOT USE

Use autoclave ID \_\_\_\_\_  
Located in \_\_\_\_\_  
until further notice.

## SERVICE REQUESTED

Contact EHS for Questions  
402.472.4925

*Reference autoclave ID# when calling*