ENVIRONMENTAL HEALTH AND SAFETY
University of Nebraska-Lincoln

Safe Operating Procedure

(Created 2/20)

AUTOCLAVE PERFORMANCE TESTING

Introduction

As part of a campus-wide program, autoclaves that are used to sterilize biohazardous waste are enrolled in the EHS "Autoclave Testing 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. This testing is needed because autoclave indicator tape **does not** prove decontamination effectiveness. Indicatory 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 used to sterilize waste from BSL-2 labs, and white labels are placed on autoclaves used by BSL-1 labs. 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:	**Beadle Center**
ID#:	**E129-2**
If this unit is moved or replaced, please contact EHS at 402.472.4925.	

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

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

EHS distributes test kits that contain the following: test organism ampoule (Geobacillus stearothermophilus), shipping tube, documentation form, and instructions. These kits are sent out at regular intervals depending on the containment level of the lab(s) that utilize the autoclave for biohazardous waste decontamination. (BSL-2 = monthly; BSL-1 = every 6 mo.)

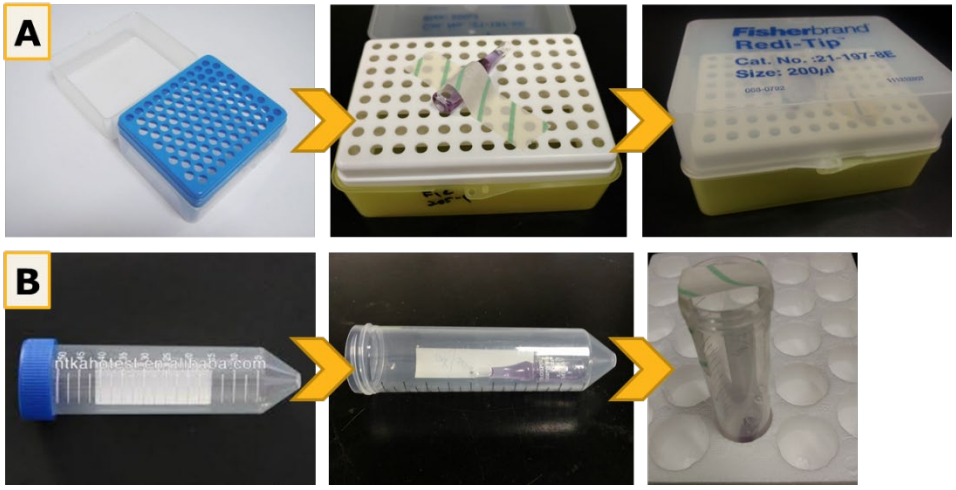
Testing Procedure

1. Open envelope and remove Falcon (50mL conical) tube and documentation form. If the packing material appears wet, 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.
4. To perform the test, use the following procedures 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 1 Autoclave Biological Indicator Testing Methods



4.2 **For liquid loads**, using a piece of string tied around the ampoule, suspend the ampoule in the liquid and secure it to the outside of the container with autoclave tape.

4.3 **For tabletop and top-loading portable autoclave/sterilizers**, (Figure 1B) (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.

IMPORTANT: 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).

4.4 **For plant material and soil in bags,** use either method 4.1 or 4.3 outlined above and shown in Figure 1.

5. Autoclave using the appropriate cycle/settings (*a typical decontamination cycle is 30-60 min at 121°C and 15-17 psi*). For difficult loads, it may be necessary to increase sterilization time or temperature. See EHS SOP, ***Autoclave Operation and Use***.
6. Allow time for the autoclave to cool down and for pressure to return to atmospheric.
7. Using insulated gloves or mitts, remove load from autoclave.
8. 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. 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

☑ **Completed documentation form**, including:

- ❑ Autoclave run parameters
- ❑ Name of PI
- ❑ Name of person performing test
- ❑ EHS autoclave ID number (from sticker on autoclave)
- ❑ Date autoclave tested
- ❑ Autoclave location

☑ **Copy of printout(s) from autoclave**.

☑ **Falcon tube with tested ampoule in packing material.**

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.*

10. Place the return address label on envelope.
11. Place in campus mail. The envelope **SHOULD BE RETURNED** to EHS by the 15th of each month.

Testing Results

EHS will incubate test ampoules and check for growth. If the autoclave is functioning properly, no growth will be observed and EHS will send a new ampoule to the lab at the beginning of the following month, for BSL-2 labs or in six months, for BSL-1 labs. If the ampoule shows signs of growth, the test has failed and EHS will arrange for a confirmatory test.

If the second test also fails, place an "**Out of Order, Do Not Use!**" sign clearly on the autoclave and submit a repair order. Be sure to indicate an alternate autoclave to use until repairs are made. A sample sign is included below. Print the sign in color, fill in the blanks and post on the autoclave in need of repair.

AUTOCLAVE
OUT OF ORDER
DO NOT USE

Use autoclave ID _____
Located in _____
until further notice.

SERVICE REQUESTED

Contact EHS for Questions
402.472.4925