UC RIVERSIDE | Environmental Health & Safety

# Procedure to Ensure Autoclave Effectiveness

Autoclaves are used to sterilize biological waste products prior to disposal in regular trash. These devices must be tested to ensure that sterilization performance meets the regulatory requirements.

There are three indicators that may be used to detect the efficacy of the autoclave process:

(1) Mechanical: pressure and temperature recording devices (data loggers),
(2) Chemical: indicators that change color after being exposed to specific temperatures, such as temperature sensitive autoclave tape. The color changes upon exposure to the given temperature, and
(3) Biological: vials of *Bacillus stearothermophilus* spores, due to the spores' resistance to heat, are used to ensure that the autoclave process has sterilized the biological products.

**Introduction:**

- ❑ Biological spore testing is performed monthly on all research autoclaves used for biological waste sterilization, and results are required to be recorded on a monthly autoclave testing log form. Log records should be kept for a minimum of three years.
- ❑ Obtain biological indicator vials, free of charge, from Environmental Health and Safety by emailing the Biosafety Officer at ehsbiosafety@ucr.edu.

**Autoclave Testing Process:**

- ❑ It is recommended that at least two spore vials (*Bacillus Stearothermophillus*) are used per test.
- ❑ Label the two spore vials with the proper information such as date, ID (test vial), etc.
- ❑ Place the test spore vials in the center of a biohazardous bag (labeled dummy bag) filled to a similar size and with similar items without any biological materials as the actual waste bags that will be disposed. **Do not place inside actual waste bags** to avoid potential exposure to biohazardous materials during retrieval of vials if autoclave process fails.

**Biohazardous bags should be loosely closed (not completely sealed) with autoclave tape or twist ties to allow for steam penetration.**

- ❑ Select appropriate cycle and run it to process the load.
- ❑ Once the cycle is completed, allow autoclave to decompress and cool down (minimum 15 minutes).
- ❑ Remove the dummy bag from autoclave and allow it to further cool down (10 - 15 minutes).
- ❑ Retrieve the spore test vials from the dummy bag.
  Note: the chemical indicator tape changes from white to black when exposed to steam.

**Incubation process:**

- ❑ Use an adjusted incubator set to a temperature of 131 – 140 degrees Fahrenheit (55 - 60 degrees Celsius).
  - Take both test vials, gently squeeze the bottom of the plastic vials to break the glass ampule within and then place the vials in the incubator.
  - Take a third spore vial (labeled as “control”) that was not run in the load. Proceed to break the glass ampule within and also incubate in the same incubator. Incubate for 24 hours with periodic examination as described below.

**Interpretation:**

- ❑ Examine the vials for any color change in the media at regular intervals (i.e. 8, 12, 18, and 24 hours). The presence of a color change to yellow indicates a fail test and positive bacterial growth. No color change indicates a passing test and proper sterilization of sample. **The control vial should produce a yellow color change.**
- ❑ If a fail test is confirmed run the test again and discontinue normal use of the autoclave. If the second test fails again, post a sign "**Do Not Use Autoclave**" and inform all appropriate personnel including Biosafety Officer at extension 2-4246 or Assistant Biosafety Officer at extension 2-1115. Discontinue full use of the autoclave until a service vendor has repaired and tested it for full functionality.
- ❑ If a passing test is confirmed from the first round or second round, you may proceed with regular use of the autoclave.
- ❑ Record all results in your monthly autoclave test log form.
- ❑ Ensure all used Bacillus vials are sterilized by autoclaving first before discarding or place used vials in biohazardous waste containers to be picked up by EH&S.

# Autoclave Monthly Log for Spore Tests

**Autoclave Location (Building & Room #): _____**

**Department: _____**

**Autoclave ID or Serial #: _____**

| Run Date & Time | User Name | Cycle Name (i.e. P5) | Cycle Time (min.) | Max. Temp. (°C) | Test Result (Pass/Fail) | Vial(s) Lot Number(s) | Comments |
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To reduce paper waste, use the same log print out to record monthly tests until all rows are used.

Email a copy of this log to the Biosafety Officer at ehsbiosafety@ucr.edu after each monthly biological indicator test.

Keep all logs for a minimum of 3 years.