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 requirements.

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

(1) Physical: pressure and temperature recording devices,

(2) Chemical: indicators that change color after being exposed to specific temperatures, such as temperature sensitive tape. The color change upon exposure to the given temperature, and

(3) Biological: *Bacillus stearothermophilus* spores are used, due to its resistance to heat, for the testing that measures the biological performance of the autoclave process.

Introduction:

- ❑ Biological spore testing is performed monthly on all research autoclaves used for biological waste, and results will be recorded on a monthly autoclave log form. Log records should be kept for a minimum of three years.
- ❑ Obtain biological indicator ampules, free of charge, from Environmental Health and Safety by emailing the biosafety officer at ehslabortory@ucr.edu.

Autoclave Testing Process:

- ❑ It is recommended that at least two spore ampules (*Bacillus Stearothermophillus*) are used per cycle.
- ❑ Label the spore ampules with the proper information. Such as date, autoclave number, etc.
- ❑ Place the spore ampoules in a horizontal position inside a Biohazardous bag with representative materials to be sterilized.

  (**Biohazardous bag should be closed with autoclave tape**)
- ❑ Select appropriate cycle to process the load.
- ❑ Once the cycle is completed, allow autoclave to decompress and cool down (min.10 minutes).
- ❑ Remove the load from autoclave and allow it to further cool down (10 - 15min).
- ❑ Retrieve the spore test ampoules from the load.
- ❑ Note: the chemical indicator tape changes from white to black when exposed to steam.

Incubation process:

- ❑ Use an adjusted microbiological incubator to a temperature for 131F – 140F (55C to 60C).
  - Take both ampoules from the loads that were autoclaved, gently squeeze the plastic crusher to break the glass ampule and place those in the incubator.
  - Take a third spore ampule (labeled as “control ampule”) that was not run in the load. Proceed to break the glass ampoule and also incubate in the same incubator, allow incubation and verify all three spore ampoules after 24 hours.

Interpretation:

- ❑ Examine the indicator ampoules for any color change after 8, 12, 18, and 24 hours. The presence of yellow color change indicates a fail test and positive bacterial growth. No color change indicates a passing test and proper sterilization of sample. **The control ampoule 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 "**Autoclave Not In Use**" and inform all appropriate personnel including Biosafety Officer at Ext: 2-2648 or Assist. Biosafety officer Ext: 2-4244. Discontinue full use of the autoclave device until approved for use.
- ❑ 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 log form.
- ❑ Ensure disposal of all used Bacillus ampoules by autoclaving first before discarding.

Autoclave Monthly Log for Spore Tests

Location:

Department

Autoclave: Serial #

AUTOCLAVE LOCATION____							
Run Date/Time	**Description of Waste**	**Weight (lbs.)**	**Cycle Time (min.)**	**Max. Temp. (°C)**	**Spore Test (+/-)**	**Date of Spore Test/ Initials**	**Comments**

Autoclave Log For _____

A COMPLETE entry must be made in this log for each use of the autoclave

Date/ Time	Generator Name	Location	Time at 121°C	Medical Waste	Biohazard Waste	Other: Specify	Sterilization Check Pass/Fail	Comments	Operator Name