

**Biological Indicator Spore Testing Procedure**

* Biological indicator spore testing is performed quarterly on all research autoclaves used for biological waste, sterilizing glassware or any other research related uses as outlined in the ECU Biological Safety Manual (Chapter 5, page 49).
* Without biological indicator testing, adequacy/ efficacy of the sterilization process cannot be assumed.
* Ampules containing *Bacillus stearothermophilus* are used for this testing, due to its resistance to heat, to measure biological performance.
* These ampules may be purchased from Comparative Medicine (Robin Alligood, 744-2452 alligoodr@ecu.edu,).
* These ampules may also be purchased directly from Medical Storeroom or from different vendors such a Fisher Scientific, VWR, or 3M. Make sure you are purchasing *Bacillus stearothermophilus* biological spore indicators. You can search “biological indicator” on these given sites and find results.
* OPTIONS:
	+ If you decide to buy your own ampules, and then have Comparative Medicine incubate, read and record the results their charge to you is $2.
	+ The cost is $5 if you purchase the ampules from Comparative Medicine AND have Comparative Medicine incubate, read and record the results.
* QUESTION: How many ampules will be needed per year? Most ampoules have a shelf life of 48 months. You will need one ampule per autoclave per quarter as well as one control. If a researcher has one autoclave they will need 8 ampules total per year (1 ampule per quarter + 1 control ampoule x 4(quarterly) =8).

If a lab has 3 autoclaves they will need 16 ampules for the year (1 ampule per autoclave per quarter (3x4 = 12) +1 control used for all 3 autoclaves once quarterly (conducted at the same time) 12+4=16.

* A log should be created and kept by the lab to record the results (see example below). This log will be checked during yearly biosafety lab inspections to ensure that this biological indicator testing is being conducted quarterly.
* If you purchase your own ampules and do all the incubating, reading and recording yourself, there should be a set of instructions included with the ampules, similar to these (follow protocol that comes with your specific ampules):

1. Every quarter perform the autoclave QC test.

2. Place *Bacillus stearothermophilus* vial in the center of the bag, or autoclave (if autoclave strictly used for sterilizing glassware/labware/instruments). (Do not place vial into concentrated culture materials being inactivated or other waste material that should not be re-handled).

3. Process the load using normal operating procedures.

4. Compress plastic vial to break glass ampule inside (this releases a bacterial growth media).

5. Incubate *B. stearothermophilus* at 55-60°C for 24-48 hours (see specific protocol included with ampules).

6. Incubate a control vial that has not been autoclaved, media should turn yellow to indicate growth. If the control vial remains purple, there may be a problem with the batch of indicator vials and the test may not be valid. Repeat the run, and if the result is the same obtain a new set of test vials. Return the waste to the bin as in step 8 below and re-run with a new batch of vials.

7. If the test vial media is purple after incubating 72 hours, sterilization is successful, there was no growth.

8. If the media turns yellow the bacteria grew, sterilization failed.

a. Review the run chart to see if the physical conditions (time, temperature) were met. If they were, discontinue using the autoclave, return the waste to a biological waste bin, and contact the service provider to get the autoclave repaired.

b. If the conditions were not met, repeat the run with a new test vial.

c. Retest after the repairs are completed.

9. Record all results and retain in the Autoclave logbook (see example below).



**Autoclave Use**

Autoclaving is one of the most dependable methods for decontaminating laboratory materials or waste. Autoclaves use saturated steam under pressure to achieve high temperatures to kill microorganisms. Attaining the proper temperature for the proper length of time is essential for an effective kill.

Items for autoclaving

CAN

Cultures & Stocks

Culture Dishes & Related devices

Discarded live & Attenuated Vaccines

Contaminated soiled items (petri dishes, pipettes, gloves, paper towels)

Items for sterilization such as glassware, media, water, equipment

Infected small animal carcasses

CANNOT

Materials containing solvents, volatile or corrosive chemicals

Material contaminated with chemotherapeutic agents

Radioactive material

Heat sensitive material, lab equipment or reagents to be re-used

Packaging

• Use only APPROVED AUTOCLAVE BAGS. Autoclave bags (orange) are available from the Medical Storeroom. (Red Biohazard bags are designed for incineration, and not suitable for autoclave use)

• Containers must be heat resistant

• Do not overfill autoclave bags or containers (do not fill beyond 75 % of holding capacity)

• Do not compress material – sufficient space is required to ensure steam penetration

• Dry material should be separated from liquid material

• If outside bag is contaminated, apply a second autoclave bag before removal from lab

(Orange Autoclave bag in plastic container; autoclave labeling not visible)

Transportation

• Surface decontaminate prior to transport

• Use cart (metal or hard plastic) with guard rails

• Use secondary containers

• Flasks must be capped with at least aluminum foil

• Use direct but not heavily populated route

• Bags should be closed for transport and loosely sealed or taped. Do not seal airtight. This prevents spillage of contents but prevents “popping” of tightly sealed bags.

Cycle Instructions

• Operate at a minimum of 250oF (121oC) for a minimum of 30 minutes.

o Cycles will vary depending on the biological hazard and size of load.

Loading Autoclave

• Ensure material is acceptable for autoclaving

• Separate Similar Loads

• Use Secondary Containers

• Load material to ensure steam penetration

• Ensure all containers & bags are well vented

Unloading Autoclave

• Wait until chamber pressure gauge reaches 0 before opening the door

• Open door slightly to allow any remaining steam to dissipate

• Remove waste safely wearing personal protective equipment

• Verify heat sensitive tape has changed color. This indicates that proper temperature has been achieved on the package surface. It does not indicate sterility of the inner contents.

Safety Considerations

• Wear personal protective equipment (heat resistant gloves, goggles, and a lab coat)

• Use caution when opening the autoclave door. Allow superheated steam to exit

• Use caution when handling a bag in case sharp objects have been inadvertently placed in the bag. Never lift a gab from the bottom of the bag to load the chamber. Handle the bag from the top.

• Watch out for pressurized containers. Super heated liquids may spurt from sealed containers. Never seal a container of liquid with a cork which may cause a pressurized explosion.

• Agar plates will melt and the agar will become liquefied. Avoid contact with this molten liquid.

• Glassware may crack or shatter if cold liquid comes in contact with this superheated glassware. If glass breaks in the autoclave, use tongs, forceps, and other mechanical means to recover fragments.

Autoclave Testing

• All autoclaves used for infected waste decontamination should be regularly monitored for effectiveness in three areas: mechanical, thermal, and biological performance

• Autoclave indicator tape indicates that the outside of the container came to temperature, it does not reflect time or conditions inside the load and does not prove effective decontamination

• General mechanical maintenance will be conducted on an annual basis by Facilities Services or as recommended by the manufacture

• Research autoclaves should be tested quarterly with a spore indicator for biological effectiveness and the results documented by user (See Autoclave Validation Policy).

o Spore indicators should be placed at the slowest point of heating (e.g. the center of the load)

o For researchers who autoclave infectious waste, it is suggested that a simulated load of typically autoclaved materials which is not infectious/contaminated with a spore indicator be run each quarter as a validity check. This load can be safely dissembled after autoclaving to check the spore indicator without risk of infectious exposure.

Safety Maintenance

• A safety check should be completed prior to each use. (e.g. door closes and seals properly, rack is in place, correct setting are being used, the interior is clean)

Record Keeping

• Daily Autoclave Log

o The use of an autoclave logbook is recommended for each autoclave. Prior to autoclaving any items, users fill in all required information. (Users Name, cycle time, cycle setting, time in/out, verification of results)

AUTOCLAVED WASTE GUIDELINES

(Treating Infectious or Potentially Infectious Waste)

What is an autoclave?

An autoclave, or steam sterilizer, is an insulated pressure chamber in which saturated steam is used to elevate the temperature. Autoclaves are found in research, diagnostic and microbiology laboratories, health centers and other places that require high-level disinfection.

How does an autoclave work?

An autoclave uses pressurized steam to decontaminate infectious waste. Laboratory autoclaves normally operate at a temperature of 250°F (121°C), a pressure of 15 pounds per square inch (psi) and a minimum cycle time of 30 minutes. The effectiveness of an autoclave depends on the time, temperature and direct steam contact with infectious agents. Other factors that influence treatment efficiency include waste density, physical state and size and organic content.

How do I use the autoclave?

• Autoclaves come in many different styles. Therefore, always follow the manufacturer’s instructions when using the autoclave and ask your supervisor if you have questions.

• There are different autoclave and exhaust cycles for liquids and solids.

• Loosely close the bag or vessel; do not tie the bag or seal the vessel tightly before autoclaving. Sealed containers can explode.

(NOTE: All autoclaves should be validated by spore testing: Weekly for healthcare use, quarterly for research use.)

What must be autoclaved?

The following human and animal infectious waste products must be autoclaved:

• Cultures and stocks of infectious agents.

• Laboratory wastes that were exposed to infectious agents from medical, pathological, or pharmaceutical laboratories.

• Pathological wastes, including tissues, and biopsy specimens.

• Research animal tissues/carcasses infected with pathogenic organisms, or biotoxins weighing less than five pounds. (All research animals must be disposed according to ECU ACUC and Department of Comparative Medicine procedures. Return to coolers in appropriate containers designating hazard status as instructed.)

• Biological waste and discarded materials contaminated with excretion, exudates, or secretion from humans or animals.

• Discarded equipment and utensils which have been in contact with pathogenic infectious agents concentrated cultures of any organisms or biotoxin.

• Preparations made from genetically altered living organisms and their products.

(See Infectious Waste flow sheet pg. 46 for additional detail)

What cannot be autoclaved?

Types of waste that should not be autoclaved include cancer drugs, toxic chemicals, radioisotopes, volatile chemicals or any other harmful material that can be vaporized and disseminated with heat. In general, do not autoclave flammable, reactive, corrosive, toxic or radioactive materials.

Preserved specimens (i.e. formaldehyde) must not be autoclaved; they can be put into the biohazard waste stream (if the excess liquid preservative has been removed). (Contact Environmental Health and Safety for liquid formaldehyde/formalin disposal). Use sharps containers for disposal of stained or preserved specimens on glass slides

How should I collect and dispose the waste?

Liquid infectious waste (excluding the chemicals listed above) may be autoclaved and then disposed via the laboratory drainage system. Or the leak-proof, labeled container may be autoclaved and then sealed and placed into a waste cart for pickup. (Do not pour melted agar into sink or floor drains. Allow it to cool and solidify for disposal as a solid waste.)

Follow these disposal procedures for solid infectious waste: (See flowchart to determine if autoclaving is needed)

1. For sharps (like needles, scalpels, pipette tips or breakable glass materials like slides, glass tubing or vials): collect the infectious waste in a puncture-resistant Red Biohazard plastic box or bin. \*Cardboard boxes may be used for disposal of sharps which are dry (no associated liquid) and which do not required autoclaving.\*

2. Use plastic red biohazard bag for collection of soft or non-sharp materials (used gloves, paper towels or plastic ware). Bags should be stored inside a plastic or metal receptacle labeled and lidded when in use in the lab.

3. Waste that does not need to be autoclaved should be placed directly in a red bag and sealed for pickup.

4. Soft solid waste to be autoclaved should be collected in orange autoclave bags.

a) The orange bag should be placed in an autoclave-resistant tub and autoclaved by the user, cooled and then placed into a new red outer bag and sealed for pickup.

b) The ECU waste technicians have been trained to recognize orange autoclave bags placed into a red bag as “autoclaved”. (They will not pick up orange bags unless placed into a red bag.)

5. Ultimately only sealed sharps containers and waste in sealed red biohazard bags should be placed into the biomedical waste cart. Contact hazardous waste technician for information regarding pick up process at 744-DUMP.

Questions?

Contact Biological Safety at 744-2237 or 744-3437.

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