

DRI Guidelines for Autoclave Use

Introduction

Materials from microbiology, biomedical, and all laboratories handling materials that may be considered biohazardous, including contaminated equipment and labware must be rendered non-infectious prior to washing, storage or disposal, even if they are not classified as biohazardous or medical wastes. An autoclave that uses saturated steam under pressure has over the years become the generally-accepted method for inactivation of all microbes.

Operational standards require that the autoclave reach a temperature of not less than 121° C (250° F) for 30 minutes at 15 pounds per square inch pressure; or in accordance with manufacturer's directions. A variety of factors can affect the efficiency of an autoclave, therefore, when treating biohazardous wastes, it is recommended that 115°C be reached and maintained for a minimum of 20 minutes within the waste itself. Biohazard waste that has been autoclaved within these standards is considered to be no longer biohazardous and is considered solid waste for disposal purposes.

Purpose and Scope

To ensure health and safety, all biohazardous materials and items potentially contaminated with infectious agents must be decontaminated prior to disposal. In addition, items that could be mistaken for medical or biohazardous (infectious) waste (agar plates used to grow non-pathogenic microbes, for example) or items that have come in contact with biological materials should be autoclaved prior to disposal.

Responsibilities

It is the responsibility of the principal investigator for each lab that uses an autoclave to develop lab specific procedures for each autoclave/steam sterilizer for which they are responsible. The procedure must address *each* of the following:

- Time
- Temperature
- Pressure
- Type of waste
- Type of container(s)
- Closure on container(s)
- Pattern of loading
- Water content
- Maximum load quantity

This standard operating procedure (SOP) outlines the elements that should be considered and included as appropriate in lab specific autoclave procedures. This lab procedure should also include a means to ensure that training, recordkeeping, and testing is conducted for each autoclave in their labs or used by their lab personnel. All personnel using autoclaves must be adequately trained by their PI or lab manager. Never allow untrained personnel to operate an autoclave.

Each individual working with biohazardous materials is responsible for its proper disposition.

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Recommended standard practices for using an autoclave are:

- Review the operator's manual for instructions prior to operating the unit. Different makes and models have unique characteristics. Never exceed the maximum operating temperature and pressure of the autoclave.
- Wear the appropriate personal protective equipment (safety glasses, lab coat and heat-resistant gloves) when loading and unloading the autoclave. Be especially careful not to stand too close when opening an autoclave. Often a pulse of hot steam escapes when the hatch is opened.
- Place autoclavable bags containing waste in a secondary containment vessel to retain any leakage that might occur, never place autoclave bags directly on the autoclave chamber floor. The secondary containment vessel must be constructed of material that will not melt or distort during the autoclave process. (Polypropylene is a plastic capable of withstanding autoclaving but is resistant to heat transfer. Materials contained in a polypropylene pan will take longer to autoclave than the same material in a stainless steel pan.)
- Use heat-sensitive tape or other device to visually check that optimal temperatures have been achieved on each container that is processed. If biohazardous or medical waste is being processed, the biological indicator *Bacillus stearothermophilus* (e.g. the Prospore Self-Contained Biological Indicator, Raven Biological Laboratories, available through Fisher Scientific, Cat. # 12-001-1) should be placed at the center of a load processed under standard operating conditions, at least monthly, to confirm the attainment of adequate sterilization conditions (see Autoclave Quality Assurance Program).
- Position autoclave bags with the neck of the bag taped loosely and leave space between items in the autoclave bag to allow steam penetration. Never place sealed bags or containers in the autoclave. Polypropylene bags are impermeable to steam and should not be twisted and taped shut.
- Select the appropriate cycle: liquid cycle (slow exhaust) for fluids to prevent boiling over, dry cycle (fast exhaust) for glassware, fast and dry cycle for wrapped items. After the cycle is complete, allow liquid materials inside the autoclave to cool down for 15-20 minutes prior to their removal to prevent boiling over.
- Autoclaving items containing solvents, volatile or corrosive chemicals is prohibited.
- Ensure that the pressure of the autoclave chamber is at zero before opening the door. Stand behind the autoclave door and slowly open it to allow the steam to gradually escape from the autoclave chamber after cycle completion.
- Never leave an autoclave in operation unattended (do not start a cycle prior to leaving for the evening).
- If operational problems occur they should be reported to the lab PI so requests for repairs can be initiated.
- Obtaining warranties and preventative maintenance plans are strongly recommended.

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Structural Inspection

Autoclaves are pressure vessels, which are regulated under NRS 455C. They must be regularly maintained and repaired by qualified technicians per the manufacturers recommended schedules. Additionally, **Nevada State law requires that autoclaves with an internal volume greater than 5 cubic feet be inspected annually by a certified inspector.** This service is currently performed at no cost to NSHE institutions by Hartford Steam Boiler. Please contact NSHE Risk Management, 775-784-4901, to arrange for an autoclave inspection.

Recordkeeping

Recordkeeping is best maintained by the individual users of the autoclave. Designate a "contact" who must:

- Test and assure proper operation of the unit
- Notify EH&S when an autoclave is removed or installed
- Notify users when an autoclave is not functioning properly

Report the contact's name to EH&S and post it near the autoclave unit.

A user log should be attached to, or near, the autoclave and must be completed by operators for each sterilizing cycle. Date, time, temperature, pressure, contact time and operator must be recorded. This log must be kept for a period of not less than one year.

Whenever necessary, operators must maintain records and procedures specified for temperature monitoring, chemical integrator monitoring, and biological indicator monitoring. These records are also kept for a period of not less than one (1) year.

Many autoclaves are equipped with a recording device that automatically records this information. In this case, the tape strip should be initialed by the operator. This record must also be kept for at least a year.

Training

An autoclave training program must be developed and implemented. All users must be trained before operating an autoclave and written operating procedures must be readily accessible.

Autoclave Quality Assurance Program

An autoclave quality assurance program is required in Washoe County for treating biohazardous waste and strongly encouraged at other DRI locations. To ensure that biohazardous waste is properly decontaminated during autoclaving, the following procedures must be followed.

- Infectious waste must be treated in an autoclave for a **minimum of 30 minutes at 121 °C (250°F)**; however, the total processing time required to decontaminate infectious waste depends on the specific loading factors (container type, water content, quantity, etc.). A total processing time of 60 minutes is recommended for gravity displacement autoclaves and 10 minutes for vacuum-type autoclaves (132 °C).

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- Sterilization by autoclaving is accomplished through exposure and penetration of the contaminated material by superheated steam for an adequate amount of time. Since steam will not penetrate a sealed plastic autoclave bag, bags containing dry loads must not be tightly sealed (rubber band closures will allow bags to “breathe”) or adequate amounts of water must be added to the load. Consult the manufacturer’s instructions for sterilizing materials inside plastic autoclave bags. Liquid waste and fresh animal carcass waste may be autoclaved inside a tightly sealed bag.
- All autoclaved waste must include a steam sterilization indicator (the use of biohazard bags with a “built-in” indicator is recommended).
- The operating temperature of the autoclave must be verified for each run by maintaining a record of the temperature either as a chart or paper tape recording or a manual recording in a logbook.
- Confirm on a monthly basis (see below) that adequate sterilization conditions are being met through the use of ampoules containing heat resistant spores (*Bacillus stearothermophilus*) placed in the center of an autoclave load. In conjunction with the *B. stearothermophilus* testing, measure and record the maximum temperature achieved during the autoclave cycle through the use of a maximum registering (or “holding”) thermometer on an Autoclave QC Log.
- Maintain records of *B. stearothermophilus* testing and maximum autoclave temperature recordings for a minimum of one year.

Monthly Spore Testing Procedure

1. Place ampoule of *B. stearothermophilus* spores and holding thermometer in the center of an autoclave load.
2. Process the load under normal operating procedures.
3. The highest temperature indicated on the holding thermometer is entered on the Autoclave QC Log. If this temperature is less than 121 °C, the autoclave is not to be used to treat infectious

Definitions

1. **Biohazardous Waste**¹—Waste which, because of its characteristics may cause, or significantly contribute to an increase in mortality or an increase in serious irreversible or incapacitating reversible illness; pose a substantial hazard to human health or the environment when improperly treated, stored, transported, disposed of, or otherwise managed. Pathological, chemotherapy, pharmaceutical wastes and dead or diseased animals subjected to regulations by the State of Nevada Department of Agriculture are excluded from this definition.

Biohazardous waste include each solid waste or waste steam in the following list

¹ Definition from Washoe County District Health Department Regulations

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- Human Blood and Human Body Fluids or items that contain or are caked with dried blood
- Cultures and Stocks of Microorganisms and Biologicals likely to contain pathogenic organisms.
- Tissues and Other Anatomical Waste from humans
- Contaminated Animal Carcasses, Body Parts, Bedding and Related Waste from animals infected with organism likely to be pathogenic to humans
- All Sharps
- Trauma Scene Waste
- Isolation Waste
- Any residue or contaminated soil, water or other debris resulting from the clean up of a spill of any biohazardous waste
- Any solid waste, excluding hazardous or radioactive waste, contaminated by or mixed with biohazardous waste.

2. **Infectious**—Capable of causing infection.
3. **Medical Waste**--A waste that meets the definition of both sharps waste or biohazardous waste (as identified above) AND is generated or produced as a result of any of the following actions:
 - a. Diagnosis, treatment, or immunization of human beings or animals.
 - b. Research pertaining to the diagnosis, treatment, or immunization of human beings or animals.
 - c. The production or testing of medicinal preparations made from living organisms and their products, including, but not limited to, serums, vaccines, antigens, and antitoxins.
4. **Sharps**--A sharp is any item having corners, edges, or projections capable of cutting or piercing the skin.

Biohazardous Sharps means any object likely to be contaminated or may become contaminated with a pathogen through handling or during transportation and also capable of cutting or penetrating skin or a packaging material. Sharps includes, but is not limited to, needles, syringes with needles, scalpels, broken glass, culture slides, culture dishes, broken capillary tubes, broken rigid plastic, Pasteur pipettes, and similar items having a point or sharp edge or that are likely to break during transportation and result in a point of sharp edge.

"Clean" Broken Glass - Use a broken glass box for clean broken glass disposal. Boxes must be heavy cardboard lined with a heavy plastic liner and marked 'Broken Glass". Once full, boxes are taped shut and put into the dumpster.

Chemically Contaminated Sharps--Sharps grossly contaminated with hazardous chemicals should be contained in a tight sealing hard sided container and labeled and handled according to DRI's hazardous waste requirements.

Radiologically Contaminated Sharps--contain in a disposable sharps container that is labeled and managed according to UNR's radiological waste requirements.