

Autoclave Standard Operating Procedure

Purpose: The purpose of this standard operating procedure (SOP) is to ensure that all potentially infectious waste materials, and waste that must be made biologically inactive before disposal, are adequately sterilized when subjected to autoclaving. This SOP will outline procedures for waste collection, treatment by autoclaving, validating autoclave performance, and a protocol for managing an autoclave that is not operating correctly (if a positive biological indicator is generated during testing of performance).

Scope: This SOP applies to all autoclaves that are used to decontaminate infectious waste materials and plant (and plant associated) materials that must be made biologically inactive prior to disposal into the normal solid waste stream. Post this SOP in a visible location near autoclaves that are used to decontaminate these types of wastes.

Responsibilities:

Users: All users are responsible for operating the autoclave in accordance with the parameters outlined in this SOP when the autoclave is being used to decontaminate or inactivate materials. Users are also strongly encouraged to run monthly tests to ensure proper performance of the autoclave.

Department/Facility Supervisors: Responsible for maintaining autoclaves in good working order and having autoclaves tested annually by a qualified technician.

The University Police and Safety Office: Providing information on autoclave testing materials and training and guidance on effective decontamination methods to the campus community.

Definitions:

Potentially infectious waste, which requires autoclaving, includes the following:

Biological waste: includes blood and blood products, excretions, exudates, secretions, and other body fluids that cannot be directly discarded into the municipal sewer system, and waste materials saturated with blood or body fluids.

Culture and stocks: includes etiologic agents of disease and associated biologicals, including specimen cultures and dishes and devices used to transfer, inoculate and mix cultures, wastes from production of biologicals, and serums and discarded live and attenuated vaccines.

Gloves and other disposable personal protective equipment used as barriers when handling biological wastes, cultures, or stocks.

Plants, plant associated materials, and plant pests may require autoclaving to render these materials biologically inactive prior to disposal. This is a condition of most APHIS permits.

Required materials:

1. Autoclavable bags
2. Solid walled holder for autoclave bag during waste collection
3. Black plastic bags to dispose of autoclave bags in once they are decontaminated to send to the landfill (red or orange biohazard bags cannot go to the landfill)
4. Shallow tub or tray to autoclave bags in (metal preferred, but can be autoclavable plastic: check with manufacturer if unsure)
5. Autoclave indicator (heat-sensitive, lead free autoclave indicator tape, Chemitest temperature verification strips)
6. Biological indicator (commercially available; contains spores; example BT Sure test vials)

Procedures:

Waste Collection:

- All potentially infectious waste must be collected into a solid walled container marked with the universal biohazard symbol. Non-infectious waste (such as plant material that requires autoclaving) may be collected in bags that are not marked with the biohazard symbol. Do not mix potentially infectious waste with non-infectious waste at any time.
- Do not overfill your waste container. Prepare the autoclavable bag by cinching, twisting, and securing the bag closed when it is no more than $\frac{3}{4}$ full.

Autoclaving:

- Place bag to be autoclaved into a shallow pan or tub.
- Affix a small piece of autoclave indicator tape or test strip to the outside of the bag.
- Place the pan and bag inside the autoclave. Do not overload the autoclave. There should be at least 2 inches of space around each waste bag on all sides to allow access to surfaces by steam. No other materials should be autoclaved together with waste in the same load.
- Run the autoclave at a chamber temperature of 121°C for 60 minutes*, using a dry cycle run.

*121°C is a standard temperature for autoclave operation, and generally achieved when chamber pressure is 15-16 psi. However, this pressure is dependent upon altitude. At higher altitudes, the pressure must be increased to achieve 121°C. It is also important to recognize that the parameters of time and temperature have an inverse relationship. Operation at higher temperatures will allow the time to be decreased, and operation at lower temperatures will require longer times. The 60 minute time specified in the directions is recommended as a starting point, but must be validated by testing as described in the later "Validating Autoclave Performance" section and adjusted if needed.

- When the cycle has been completed, verify that the autoclave chamber and ambient pressure are the same. The chamber may now be opened and the waste bag removed. **Wear autoclave gloves when handling hot items.** Also use caution when opening the autoclave door, as a small amount of hot steam may be released when opening the chamber.

- Verify that the cycle ran appropriately by visualizing the heat indicator tape or strip.
- Once the bag has cooled, place the treated waste bag inside a black plastic bag and close the bag either by knotting or with a twist tie. The treated waste may now be discarded as normal solid waste.
- Please be sure and select the most appropriate cycle for rendering biohazardous materials non-viable for disposal. The above instructions are for solid waste, however if the materials you need to autoclave are liquid cultures, be sure to use the liquid autoclave cycle to avoid creating a mess within the autoclave due to overflow of liquid from the container(s). Always use secondary containment for liquids when autoclaving such as a shallow pan or tub.

Validating Autoclave Performance:

- Each autoclave must have a functional monitoring or measuring device (electronic or dial) to ensure that the recommended temperature is achieved for the proper length of time on each load.
- Each waste bag or container decontaminated by autoclaving should have a heat sensitive indicator such as autoclave tape or strip attached to the outside of the bag. These should be visualized before disposal of each bag and should remain with the bag.
- At least once a month, autoclaves that are used to decontaminate waste or to render materials biologically inactive should be tested by using a biological indicator, such as endospores from the bacterium *Geobacillus stearothermophilus*. Testing procedure:
 - Secure a biological indicator test containing endospores such as BT Sure (available from The Safety Office at no cost, or from both Fisher Scientific and VWR)
 - Tie a piece of string to the testing vial containing spores to facilitate retrieval of the vial after the autoclave run. Add vial containing spores to the bag of waste, burying it within the waste (see Precautions section of this SOP about reducing potential exposures during this step). Leave the other end of the string attached to the vial trailing out of the opening of the waste bag.
 - Secure the waste bag and start the autoclave run.
 - Post autoclaving and once the bag has cooled, retrieve the vial.
 - For BT Sure indicators: To activate the media, with gloves on hold the indicator in an upright position, gently squeeze to break the glass ampoule.
 - Follow incubation instructions to complete the test. For the BT Sure vials, this is 55-60° for up to 48 hours.
 - Read the results of the indicator according to manufacturer instructions. For BT Sure vials this is a color indicator and reads as follows:
 1. If after 24 hours the media is yellow this = a failed test (the endospores grew, they were not killed).
 2. If after 24 hours the media is still purple = presumptive pass, but continue to incubate until 48 hours.
 3. If after 48 hours the media is still purple = passed test (all endospores were killed).
 4. If spores survived the autoclave process, growth will lead to fermentation and the production of acid turning the media yellow.

- Record date, run parameters, autoclave tested, and test results (a sample record sheet is included in on page 5 of this SOP).
- The Safety Office will supply, incubate, and read the results of the indicator for you if you prefer. If you would like the Safety Office to assist you with validating performance of your autoclave, follow this protocol:
 1. Obtain a biological indicator from the Safety Office by calling 1-7759. Please include the name and the room number you would like the indicator sent to (via campus mail).
 2. Run the indicator in a typical waste load. Remove the indicator following the precautions included in this SOP (page 4). Place the indicator in the supplied Ziploc bag.
 3. Fill out the applicable portion of the form that arrived with your indicator. If you have lost the form, you can download a new form from the [NDSU Downloadable Forms Page](#) (under Safety-Biosafety)
 4. Place the bagged indicator and the filled out form in a campus mail envelope and mail to the University Police and Safety Office
 5. The Safety Office will incubate the indicator for you, inform you of the results, and keep a record of your autoclave performance.
- If you are testing a liquid cycle: You can use the B/T Sure Indicators to validate the autoclave liquid cycle, however do not submerge the B/T Sure Indicators in liquid during the autoclave cycle. Simply run the B/T Sure Indicator in the chamber alongside what would be a typical autoclave liquid cycle run (with a normal number of tubes, flasks, etc.) for you or your lab. If you'd like to run indicators submerged in liquid, these types of indicators do exist and you can find options on the [Fisher Scientific](#) website. The Safety Office does not provide these types of indicators.
- Understand the limits of testing:
 - Testing with a temperature indicator (tape or strip) only lets you know if the autoclave reached the approximate desired/operating temperature, but will not tell you how long that temperature was maintained.
 - Using a recording device or computer to track time and temperature will not ensure that the materials inside the center of your waste bag have been sterilized.
 - Validating performance using a biological indicator is the only way to ensure complete inactivation/sterilization.
- Precautions when working with autoclaves:
 - Always wear thermal protective gloves when handling items that have been recently autoclaved.
 - Use caution when opening an autoclave door. Always verify that the chamber pressure has come down to 0 psi before trying to open the door. Still, a little bit of steam may be released when you open the autoclave door.
 - Be cautious about minimizing exposure during placement and retrieval of the testing vial. At a minimum use personal protection equipment (PPE) such as gloves, safety glasses, and a lab coat and mechanical methods such as forceps to avoid exposures. If you are generating high risk waste that presents a risk for potential exposure, the vial may be run inside a bag of waste that has previously been autoclaved.

- The following items should not be autoclaved:
 1. Polyethylene plastics (LDPE and HDPE)
 2. Solutions or waste products that contain chemicals characterized as corrosives (this includes bleach), solvents, flammables, volatiles, or radioactive materials
 3. Anything in a sealed container

Recording and Documentation

Records of repairs, service calls, and calibrations of autoclaves should be maintained by the user, facility manager, or the department.

Users must maintain records of any validation testing they perform on the autoclaves. These records should be also be kept for the lifetime of the autoclave, or as long as feasible. If you have the Safety Office read the indicator tests for your autoclave, results will be shared with users, and records of these tests will be maintained electronically in the Safety Office.

Autoclave Testing Record:

Test Vial Information

Test Vial #:

Test Vial Type: For example BT Sure

Test Vial lot #:

Expiration Date:

Each Vial contains: for example for BT Sure vials contain 2×10^5 *Geobacillus stearothermophilus* endospores

Incubation conditions: for example 55°C for 48 hours

Autoclave Information

Building:

Room:

Manufacturer:

Model:

Serial #:

Test Conditions

Time:

Chamber Pressure:

Type of Waste:

Date:

Results:

Name of Tester

Contact Person

Comments

Protocol for handling an autoclave that tests positive with a biological indicator:

1. Remove the autoclave from service. Notify a supervisor.
2. As soon as possible, repeat the biological indicator test in three consecutive cycles. If additional spore tests are positive, the items from the suspect load should be considered nonsterile and be reprocessed. Materials processed since the last acceptable (negative) biological indicator should be recalled/resterilized if possible.
3. Check to ensure that the autoclave was used correctly (for example verify that the correct time and temperature settings were used). If not, repeat using the appropriate settings and recall/reprocess all inadequately processed materials.
4. Check with maintenance support to determine if any irregularities may provide an explanation (electrical for example) or if there were any unexpected changes in the steam supply (from the standard $\geq 97\%$ steam and $< 3\%$ moisture). Any abnormalities should be reported to the person who performs sterilizer maintenance.
5. Check to ensure that the correct biological indicator was used, that it was not expired, and that the results were appropriately interpreted. If not, repeat the test using appropriate supplies.
6. If steps 1-5 resolve the problem, and if all three repeat biological indicators from three consecutive autoclave cycles are negative, then put the autoclave back into service.
7. If one or more biological indicators are positive however, do one or more of the following until the problem is resolved:
 - a. Request an inspection of the equipment by autoclave maintenance personnel.
 - b. Have the steam supply lines inspected.
 - c. Discuss the abnormalities with the autoclave manufacturer.
 - d. Repeat the biological indicator tests using a different manufacturer's indicator.
8. If there still is no resolution to the problem, close the autoclave down until the manufacturer can assure that it is operating properly. Retest at that time with biological indicators in three consecutive autoclave cycles.

References:

Oregon State University: Autoclaving of Potentially Infectious Waste SOP. Date 2/2007

Centers for Disease Control and Prevention Guideline for Disinfection and Sterilization in Healthcare Facilities. 2008