

# Standard Operating Procedure (SOP): Autoclave Verification for Decontaminating Biohazardous Waste

**Effective Date:** November 5, 2025

**Prepared by:** NDSU Safety Office

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## 1. Purpose

This standard operating procedure outlines the required procedures for the safe and effective decontamination of biological waste using an autoclave. It applies to all faculty, staff, and students who generate or manage potentially infectious waste in campus laboratories. By following this SOP, autoclaves used to treat biological waste will be verified to achieve adequate time, temperature, and pressure for decontamination and to ensure viable biohazardous organisms are not sent to the landfill. Note: the use of autoclave tape does not ensure decontamination, it is a visual indication only that sufficient temperature has been reached.

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## 2. Scope

This procedure applies to any campus autoclave used for decontaminating:

- Microbial cultures
  - Biologically contaminated solids and liquids
  - PPE and disposable materials used in BSL-1 or BSL-2 labs
  - Animal bedding and small instruments (if applicable)
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## 3. Definitions

- **Biological Indicator (BI):** A tube containing spores (*Geobacillus stearothermophilus*) used to confirm autoclave sterilization.
- **Chemical Indicator (CI):** A visual strip or tape that changes color to confirm heat exposure (e.g. autoclave tape).
- **BSL-1 / BSL-2:** Biosafety levels that describe lab containment practices based on biological risk.

## 4. Training Requirements

- All users must complete autoclave safety training.
  - Refresher training required annually or after incidents.
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## 5. Responsibilities

- **Lab Users:** Properly bag, label, load, monitor, and dispose all autoclave waste.
  - **Lab Managers/PIs:** Ensure training and compliance with SOP.
  - **Safety Office:** Provide and incubate biological indicators, communicate results, and conduct audits. **The Safety Office will supply one 10<sup>5</sup> BI monthly. Additional BIs are the responsibility of the lab.**
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## 6. PPE Requirements

Before operating the autoclave, users must wear:

- Lab coat
  - Heat-resistant gloves
  - Safety goggles or face shield
  - Closed-toe shoes
  - Long pants
  - Rubber apron
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## 7. Acceptable Materials for Autoclaving

Material	Autoclave?	Notes
Agar plates	Yes	Place in autoclavable tray with absorbent pad
Liquid cultures	Yes	Use secondary containment with loose lids
Contaminated solids	Yes	Place in autoclave bag, not sealed airtight
Bleach or chemical waste	<b>NO</b>	<b>NEVER</b> autoclave
Polypropylene tubes (PP)	Yes	Check for melting risk
Polyethylene plastics (LDPE)	<b>NO</b>	Will melt or deform

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## 8. Verification Procedure

## Autoclave Verification for Decontaminating Biohazardous Waste SOP

- I. **Check autoclave status:** Ensure it's in good working order.
- II. **Placement:** Place one biological indicator in the most difficult location to sterilize, usually in the middle of the waste bag/material to be autoclaved or suspended in a volume of liquid. Autoclave tape can also be used to secure the vial to the inside of an autoclave bag. Note: if placing among waste material, provide a means to retrieve the vial, e.g. a piece of string.
- III. **Prepare materials:**
  - Solid Waste:
    1. Use only **autoclave rated bags**.
    2. Do not overfill bags or the autoclave.
    3. Add **50-100 mL of water** to solid waste bags (steam penetration).
    4. Leave waste bags slightly open to allow for penetration of steam.
    5. Bags should be placed in stainless steel or polypropylene trays prior to or during autoclaving.
  - Liquid Waste:
    1. Use only borosilicate or polypropylene containers.
    2. Do not fill containers more than 75% capacity.
    3. Ensure caps or stoppers on all containers are loosened. Never autoclave sealed containers of liquid as this can result in an explosion of superheated liquid.
    4. Containers should be placed on stainless steel or polypropylene trays with ¼ to ½ inches of water in the bottom of the tray for even heating. Tray should be placed on a shelf in the autoclave not on the bottom of the chamber.
- IV. **Running the autoclave:** Select the appropriate cycle/settings and operate the autoclave according to the manufacturer instructions. Cycle settings are dependent on organism, but typically:
  - **Solid waste:** 121°C, 15 psi, 60 minutes minimum (dry cycle)
  - **Liquid waste:** 121°C, 15 psi, 30–45 minutes (liquid cycle)
- V. **Unloading:**
  - Allow autoclave to cool down and pressure to return to atmosphere for 10-15 minutes after cycle is complete.
  - Use heat-resistant gloves, apron, and face shield/goggles to remove trays carefully.
  - Handle BI with care. Failure to allow sufficient cooling time (10-15 minutes) may result in bursting of the vial! Remove the indicator from the waste bag wearing appropriate protective equipment.
  - **Wipe the surface of the BI with appropriate disinfectant and place in a Ziploc bag.**
- VI. **Incubation:**
  - Complete an [autoclave verification form](#), then contact Safety Office ([ndsu.biosafety@ndsu.edu](mailto:ndsu.biosafety@ndsu.edu)) to arrange a pickup of the BI.
  - The Safety Office will incubate per manufacturer instructions (e.g., 55°C for 24 hrs).
  - Results will be logged:
    1. **No color change (Purple) = PASS**

**2. Color change (Yellow) = FAIL**

- Safety Office will notify contact person of results within 24 hours of incubation being complete.

**VII. Recordkeeping**

- Each lab must maintain:
    1. Autoclave log (date, user, cycle used, waste type)
    2. Verification results (date, pass/fail)
    3. Training records for each user
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**9. Failed Biological Indicators**

**If a cycle fails to sterilize the BI:**

- The Safety Office will notify the contact person and/or lab supervisor.
  - Isolate all biological waste; do not dispose in the trash.
  - Identify potential causes, complete any necessary modifications and arrange to reverify the cycle.
  - If the cycle fails again, contact the manufacturer to determine if repairs are needed
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## **Appendix A: Quick Reference Guide (For Posting)**

### **Autoclave Cheat Sheet**

- OK to autoclave: agar plates, gloves, pipettes, cultures
- DO NOT autoclave: bleach, chemicals, sealed containers, sharp objects
- Cycle: 121°C, 15 psi, 60 minutes for solids | 30–45 minutes for liquids
- Use BI monthly in center of load
- Use heat-resistant gloves and face shield

For help: Contact NDSU Safety Office at [ndsu.biosafety@ndsu.edu](mailto:ndsu.biosafety@ndsu.edu) or 1-7759