

## **Autoclave Operations and Verification Standard Operating Procedure (SOP) 2025**

**Autoclave Make/Model:** \_\_\_\_\_

**Location (Bldg/Rm):** \_\_\_\_\_

**Responsible Individual:** \_\_\_\_\_

**Responsible Individual Phone Number:** \_\_\_\_\_

## Autoclave Approved User List

By signing below, I acknowledge that I have read and understood the contents of this document, and have agreed to conduct my activities in accordance with Northern Arizona University's autoclave policies. I understand that breaching these standards may result in disciplinary action, including termination of my status as an autoclave-approved user. To ensure proper and consistent training, approval is only valid if also signed by the Responsible Individual.

User Name	Date	User Signature	Responsible Individual Signature

## **Policy Statement**

It is the policy of Northern Arizona University (NAU) to provide a safe working environment. The primary responsibility for ensuring safe conduct and conditions in the laboratory resides with the principal investigator.

The Biological Safety Office is committed to providing up-to-date information, training, and monitoring to the research and academic community concerning the safe conduct of biological, recombinant, and acute toxin research and the handling of biological materials in accordance with all pertinent local, state and federal regulations, guidelines, and laws. To that end, this manual is a resource, to be used in conjunction with the federal guidelines, the NAU Select Agent Program, Biosafety in Microbiological and Biomedical Laboratories (BMBL), and other resource materials.

## **Objective**

To describe policies and procedures related to safe autoclave operation, installation of new autoclaves, routine preventive maintenance and repairs, training and verification of autoclaves. These policies and procedures are best practice recommendations; however, specifics of daily use and routine maintenance shall be performed according to manufacturers' recommendations. These policies and procedures apply to all laboratories at Northern Arizona University, which generate biohazardous waste, with the exception of laboratories operating at Biosafety Level 3, which are subject to a more stringent standard of operation.

Implementation of this program by all research departments within Northern Arizona University will ensure that all potentially infectious waste or biohazardous waste, as defined in Northern Arizona University Biosafety Manual, will be decontaminated prior to off-site treatment (i.e., autoclaving or incineration).

## **Responsibilities**

In the academic research/teaching setting, the Principal Investigator (PI) is responsible for ensuring that all members of the laboratory are familiar with safe research practices. In the clinical laboratory setting, the faculty member who supervises the laboratory is responsible for safety practices.

Autoclave users are responsible for reading this manual and carrying out the safety practices outlined here.

The Office of Biological Safety will provide guidance, information, review, monitoring, and training regarding NAU's Autoclave Program, and will perform bi-annual verification testing.

## Definitions and Acronyms

**Autoclave:** An apparatus for the sterilization of materials by steam under pressure. Autoclaving is one of the most effective methods for decontamination of potentially biohazardous waste. The amount of time and degree of temperature necessary for sterilization depends on the materials being sterilized and how they are packaged and loaded into the autoclave.

**Biohazardous Waste:** Solid and/or liquid waste that contains or has been in contact with infectious agents, potentially infectious materials, or recombinant or synthetic nucleic acids. This includes human and animal cell lines, tissue culture, blood/blood products, and associated waste (e.g., pipets, gloves, disposable gowns, tubes, tips, petri dishes). Waste for decontamination and disposal must be stored in leak-proof containers lined with red biological hazard bags that meet both Occupational Safety and Health Administration (OSHA) and American Society for Testing and Materials (ASTM) standards.

**Mixed Waste:** Waste material that is contaminated with a combination of chemical, biological or radiological hazards (e.g., amalgam (mercury), lead foil, adjuvants, chemotherapeutics, etc.). Special consideration must be given to the disposal of this type of waste and many times autoclaving is inappropriate. Please contact NAU's Environmental Health and Safety to set up a waste stream for non-autoclavable materials.

**Responsible Individual:** The individual, designated by the Department or Facility owner of the autoclave, tasked with ensuring verification, recordkeeping, preventative maintenance and training for a specific autoclave unit.

**Verification:** Process involving a biological or chemical challenge to ensure autoclaves are performing to an appropriate standard to effectively inactivate biohazardous waste.

## Other Hazards

1. **Heat burns** from hot materials and autoclave chamber walls and door.
2. **Steam burns** from residual steam upon completion of the cycle.
3. **Hot fluid scalds** from boiling liquids and liquid spills in the autoclave.

## Safety Precautions

1. Personnel may only operate an autoclave after they have been trained by the Responsible Individual in the proper care and use of autoclaves and warned of potential hazards.
2. Personnel using an autoclave must wear appropriate PPE.
3. Autoclaves must be inspected at least once annually.
4. Bi-annual chemical or biological indicator tests must be run to validate autoclave effectiveness.

# Operating Procedures

## 1. Material Preparation

- a. Wear appropriate personal protective equipment including a lab coat (or disposable gown), gloves, and closed-toe shoes.
- b. Ensure that the material is safe for autoclaving.
  - i. Solvents and substances that may emit toxic vapors should not be autoclaved.
  - ii. Avoid autoclaving bleach, if possible, as it corrodes the autoclave interior.
  - iii. Do not autoclave cracked glassware.
  - iv. Plastics must be heat resistant (i.e., polycarbonate (PC), PTFE/Teflon, and most polypropylene (PP) items).
- c. All biological waste, except sharps, shall be contained in a RED biohazard bag, which is impervious to moisture and has sufficient strength to resist ripping, tearing, or bursting under normal conditions of use and handling.
  - i. Loosely close bags and secure with autoclave. Wrapping too tightly will impede steam penetration, decreasing effectiveness of the process.
  - ii. Containers of liquid must not be more than 2/3 full; vent lids on bottles to aid steam penetration and avoid explosion due to pressure buildup.
  - iii. If autoclaving liquids or > 20 agar plates, place packaged material in a secondary container to secure and contain potential spills (i.e., stainless steel tray or autoclavable polypropylene bin).
- d. Animal carcasses and gross tissue specimens are to be autoclaved in YELLOW biohazard bags and stored frozen until they are removed for off-site incineration.
- e. Sharps shall be placed in a designated "sharps container" (rigid, red plastic) and stored as waste after autoclaving until they are removed for off-site treatment.

## 2. Loading the Autoclave

- a. Wear appropriate personal protective equipment including lab coat (or disposable gown), closed-toe shoes, and heat-resistant gloves.
- b. Items being sterilized for use (e.g., medical instruments, media) and waste should be autoclaved separately.
- c. Load material to allow efficient steam circulation (i.e., do not allow material to touch the sides or top of the autoclave chamber).
- d. Include appropriate verification materials with load as needed (e.g., autoclave tape or biological indicator).
- e. Ensure the door closes properly and securely.
- f. Choose the appropriate cycle for your materials and initiate the cycle.
  - i. The Responsible Individuals should provide training to users on which cycle is appropriate, as cycles can vary among autoclave models.
  - ii. Consult the autoclave manual for assistance in choosing a cycle. As a general rule:
    - **Liquid Cycles:** Used when autoclaving liquids (or materials that become liquid at high temperatures), such as broth, media, agar, buffer, saline, and water.
    - **Gravity Cycles:** Used for autoclaving glassware, waste in red biohazard bags, vented containers, unwrapped instruments.
    - **Vacuum Cycles:** Used for autoclaving porous materials, partially-vented containers, wrapped goods, animal cages with bedding.

- g. Record information in the Autoclave User Log for the load you are processing.  
**Note** - users must return to this log entry upon removal of their autoclaved materials to document that the run completed successfully.
- h. If problems are perceived with the run abort the cycle, place a note on the autoclave indicating that it should not be used, make a note in the Autoclave User Log, and report the issue to the Responsible Individual.

### **3. Unloading the autoclave**

- a. Wear appropriate personal protective equipment including lab coat (or disposable gown), closed-toe shoes, and heat-resistant gloves.
- b. Allow the autoclave to completely finish cycle before attempting to open the door or unload. Pressure gauge must read zero.
- c. Verify that cycle conditions were met by checking the autoclave printout or by visualization of the biological or chemical indicator.
- d. Remove the waste bags and place them in a biohazardous waste collection bin. To schedule a pick-up of full waste bins go to <https://nau.edu/research/compliance/environmental-health-and-safety/hazardous-waste-management/> and click on "Service Request" on the right.
- e. Shut autoclave door.
- f. Record results of processing in the Autoclave User Log.

### **4. New Autoclaves**

- a. Before newly installed autoclaves can be used for decontamination of biohazardous waste the following conditions must be fulfilled:
  - i. Inspection and certification as a pressure vessel.
  - ii. Initial autoclave verification:
    - 1. Calibration services should be completed by the manufacturer on all new autoclave units. Documentation of this calibration shall be maintained with the maintenance records for the unit.
    - 2. To verify calibration, the typical bi-annual biological indicator verification shall be completed prior to processing biohazardous waste in the unit.

### **5. Maintenance and Repair**

- a. Autoclaves must be inspected and calibrated annually by a professional (e.g., Getinge, CBS Tech) to meet regulatory requirements.
- b. Autoclave operation and maintenance manuals shall be maintained by the Responsible Individual and provided to service technicians as needed during preventive maintenance and repair activities.
- c. Preventive maintenance shall be performed according to manufacturers' suggested procedures.
- d. Following significant maintenance activities or repairs, an indicator verification should be completed prior to processing biohazardous waste in the unit.

### **6. Training**

- a. Autoclave training is provided by Responsible Individual or designee within the facility or department to users on standard operating procedures for usage of specific facility autoclave(s).
- b. Users must be trained for each autoclave they use, as standard operating procedures may vary among individual autoclave units.

## 7. Performance Verification

- a. Routine verification should be performed with each run and can be achieved through use of one or more of the following:
  - i. Autoclave tape
  - ii. Verification of temperature and run time via an autoclave run printout
  - iii. Biological/chemical indicator
- b. Bi-annual verification (performed by NAU's Office of Biological Safety)
  - i. Conditions (time, temperature, and pressure) used by each autoclave must be verified bi-annually using an appropriate biological indicator.
  - ii. Results shall be recorded in the Bi-annual Verification Log, listing the conditions, date, and name of person performing the test.

**Note** - If verification procedures fail, waste should be removed to an alternate autoclave which has been verified for processing until repairs are completed and the equipment is re-verified.

## 8. Incident Response

- a. All incidents, including a spill or release of biohazardous materials, must be reported to the Responsible Individual and to NAU's Office of Biological Safety.
- b. If injury occurs seek first aid or, if necessary, seek medical attention at NAU's Campus Medical Services or by dialing 911.
- c. Place a note on the autoclave indicating that it is not to be used until the cause of the incident is determined and the autoclave is deemed safe for operation.

## 9. Spill Clean-up

- a. Spills occur from boil-over of liquids or breakage of containers.
- b. The autoclave must not be operated until the spill is appropriately cleaned up.
- c. If a spill occurs, review the Safety Data Sheet(s) for the spilled materials, if appropriate, to determine necessary PPE, disinfection, and clean-up/disposal protocols.
- d. Wait until the spilled materials have cooled before attempting clean-up.
- e. Contain the spread of liquids by placing absorbent material around the spill area.
- f. Working from the outside towards the center of the spill, scoop/wipe up the spilled materials and place in the appropriate waste receptacle.
- g. Report the spill and clean-up procedure used to the Responsible Individual.

## Autoclave Use Log

<b>BEFORE RUN:</b> Date/time of run:		Tape or staple autoclave printout here:
Staff/student name(s):		
Staff/student phone number:		
Number of bags:		
Material being autoclaved (e.g., waste, media, etc.):		
Run cycle used:		
<b>AFTER RUN:</b> Autoclave tape changed color (circle):	Yes      No	Notes:

<b>BEFORE RUN:</b> Date/time of run:		Tape or staple autoclave printout here:
Staff/student name(s):		
Staff/student phone number:		
Number of bags:		
Material being autoclaved (e.g., waste, media, etc.):		
Run cycle used:		
<b>AFTER RUN:</b> Autoclave tape changed color (circle):	Yes      No	Notes:

<b>BEFORE RUN:</b> Date/time of run:		Tape or staple autoclave printout here:
Staff/student name(s):		
Staff/student phone number:		
Number of bags:		
Material being autoclaved (e.g., waste, media, etc.):		
Run cycle used:		
<b>AFTER RUN:</b> Autoclave tape changed color (circle):	Yes      No	Notes:

<b>BEFORE RUN:</b> Date/time of run:		Tape or staple autoclave printout here:
Staff/student name(s):		
Staff/student phone number:		
Number of bags:		
Material being autoclaved (e.g., waste, media, etc.):		
Run cycle used:		
<b>AFTER RUN:</b> Autoclave tape changed color (circle):	Yes      No	Notes:



## Autoclave Bi-Annual Verification Log

<b>BEFORE RUN:</b> Date/time of run:		Tape or staple autoclave printout here:
Name of individual performing verification:		
Contact phone number:		
Number of bags (if applicable):		
Indicator used in verification:		
Run cycle used:		
<b>AFTER RUN:</b> Verification successful (circle):	Yes      No	Notes:

<b>BEFORE RUN:</b> Date/time of run:		Tape or staple autoclave printout here:
Name of individual performing verification:		
Contact phone number:		
Number of bags (if applicable):		
Indicator used in verification:		
Run cycle used:		
<b>AFTER RUN:</b> Verification successful (circle):	Yes      No	Notes:

<b>BEFORE RUN:</b> Date/time of run:		Tape or staple autoclave printout here:
Name of individual performing verification:		
Contact phone number:		
Number of bags (if applicable):		
Indicator used in verification:		
Run cycle used:		
<b>AFTER RUN:</b> Verification successful (circle):	Yes      No	Notes:

<b>BEFORE RUN:</b> Date/time of run:		Tape or staple autoclave printout here:
Name of individual performing verification:		
Contact phone number:		
Number of bags (if applicable):		
Indicator used in verification:		
Run cycle used:		
<b>AFTER RUN:</b> Verification successful (circle):	Yes      No	Notes: