UNIVERSITY OF WYOMING

Standard Operating Procedure (SOP) for Autoclave Operation

Autoclave Make/Model: _____

Person Responsible and Phone #: _____

Located in Building/Room: _____

I. Purpose

The purpose of this document is to provide standard operating procedures for the use of autoclaves. Autoclaving is a process used to destroy microorganisms and decontaminate biohazardous waste and microbiological equipment used at Biosafety 1, 2, 3, and medical clinics at the University of Wyoming.

II. Risk Management

A. Potential Risks

Autoclaves use high pressure and high temperature steam for sterilization. The potential safety risks for operators include:

- **Heat burns** from hot materials and autoclave chamber walls and door.
- **Steam burns** from residual steam coming out from autoclave and materials on completion of cycle.
- **Hot fluid scalds** from boiling liquids and spillage in autoclave and during transport of superheated materials.
- **Hand and arm** injuries when closing the door.
- **Body** injury if there is an explosion.
- **Biological exposures** to biohazardous waste that might not have been processed if autoclave failure occurs.

B. Health and Safety

To ensure the health and safety of personnel using the autoclave, it is important for each department and/or principle investigator (PI) to maintain autoclaves and to train personnel in their proper use.

- The name of the person responsible for the autoclave shall be posted near the autoclave.
    - The person responsible can be a PI, supervisor, or lab manager for the department.
    - This SOP should be posted near the outside of the autoclave as well.
- It is the PI, supervisor, or lab manager's responsibility to ensure employees are trained before operating any autoclave unit.
- The autoclave shall be operated according to manufacturer's instructions and recommendations.
- Personal protective equipment (PPE) must be worn when loading and unloading the autoclave.
- Autoclaves must be inspected at least annually. Inspection services may be managed by your manufacturer's preventative maintenance contract or by a certified autoclave technician. A basic visual inspection should be performed monthly by the person responsible for the autoclave.

    - A basic visual inspection should be made by the user prior to each use of the autoclave as well.
        - Potential issues should be immediately reported to the individual responsible for the autoclave.
- Autoclave effectiveness shall also be regularly validated as described below (III-H).

C. Personal Protective Equipment (PPE)

Equipment to protect against scalds and burns include:

- Heat-insulating gloves that provide complete coverage of hands and forearms.
- Lab coat.
- Eye protection.
- Closed-toe footwear.

III. Operator Instructions

A. Training

All personnel who use autoclaves must have successfully completed a training session from the person responsible for the autoclave on the safe operating procedures. This requirement applies to both new and experienced personnel. An example of training documentation is provided at the end of this document.

B. Material Preparation

- Ensure that the material is autoclavable. Oils, waxes, some plastics, flammable materials, and samples containing solvents or substances that may emit toxic fumes should not be autoclaved.
    - Never sterilize items containing chlorides or producing chloride gases in the automatic autoclaves
    - Glassware must be heat-resistant borosilicate.
    - Plastics must be heat-resistant, i.e., polycarbonate (PC), PTFE (Teflon), and most polypropylene (PP) plastics.
- Glassware must be inspected for cracks prior to autoclaving.
    - Any cracked glassware should not be autoclaved.
- Prior to decontamination, all potentially biohazardous material shall be kept in secondary containment containers (stainless steel or autoclavable trays) to protect against leakage.
    - No potentially hazardous materials should be left on countertops or floors without secondary containment.
- Prepare and package material suitably:
- Loose, dry materials must be wrapped or bagged in steam-penetrating paper or loosely covered with aluminum foil. Wrapping too tightly will impede steam penetration, decreasing effectiveness of the process.
- Loosen all lids to prevent pressure buildup. All containers must be covered by loosened lid, steam-penetrating bung, or with aluminum foil.
    - Containers of liquid must not exceed 80% full, with lids loosened.
    - Discarded sharps must be in a designated ‘Sharps’ container.
    - Autoclave tape is recommended for each separate container in each load.
- Place items in secondary containers to secure and contain spills:
    - Items should be placed in a stainless steel pan or other autoclavable container for their stability and ease of handling.
    - Place containers of liquid, bags of agar plates, or other materials that may boil over or leak into a secondary pan in the autoclave.

        - The pan must be large enough to contain a total spill of the contents.
        - The pan must sit flat in the autoclave, i.e. no leaning or tilting of the pan.
    - Bags must not be tightly sealed as steam cannot penetrate.
- Biohazardous waste must be processed according to approved Institutional Biosafety Committee (IBC) registrations.
- All decontaminated solid biohazard must be placed in an opaque garbage bag and taken to the outside garbage receptacle by the individual responsible for that decontaminated biohazard.
    - The custodial staff does not handle decontaminated biohazardous waste.
    - All solid research materials, such as decontaminated mouse bedding, should be disposed of as decontaminated solid biohazard.

C. Loading the Autoclave

- Wear lab coat, eye protection, heat-insulating gloves, and closed-toe shoes.
- Place material in autoclave.
    - Do not mix incompatible materials.
- Do not overload; leave sufficient room for steam circulation.
    - As noted above, all autoclave pans and containers should be sitting flat and not be angled or leaning inside the autoclave.
- Close and latch the door firmly.

D. Operating the Autoclave

- Verify that the door is latched firmly.
- Choose the appropriate cycle (i.e., gravity, liquid, or dry cycle) for the material. Consult your supervisor, PI, or individual responsible for the autoclave for assistance in choosing a cycle.
- Start your cycle and fill out the autoclave user log with your contact information. A completed cycle usually takes between 0.5-1.5 hours, depending on the type of cycle.
- Do not attempt to open the door while the autoclave is still operating.
- If problems with your autoclave are perceived, abort the cycle and report it to your PI and the individual responsible for the autoclave immediately.

E. Unloading the Autoclave

- Wear heat-insulating gloves, eye protection, lab coat, and closed-toe shoes.
- Ensure that the cycle has completed and both temperature and pressure have returned to a safe range.
    - Typically this means that the temperature is below 100C and the pressure is back to 0psi.
- Wearing PPE, stand to the side of the autoclave (not directly in front of the door) and unlatch the door slowly, allowing extra steam to escape. Once door is completely unlatched, carefully open the door no more than 1-2 inches. This will release the remaining steam upwards and allow pressure within liquids and containers to normalize.
- Allow autoclaved load to stand for a few minutes in the chamber. This will allow steam to clear and trapped air to escape from hot liquids, reducing risk to the operator.
- Do not agitate containers of super-heated liquids or remove caps before unloading.
    - Do not agitate or remove caps until the liquids have cooled to a safe level.
- Wearing heat-insulated gloves, remove items from the autoclave and place them on a stable structure to allow them to cool.
    - This stable structure may be a water bath for liquids which need to remain molten.
- Shut the autoclave door and partially latch if necessary to keep the door closed.

F. Autoclave Use Log

- Entries must be placed on the lab sheet each time the autoclave is used. The records are kept for maintenance / service reasons and reporting of incidences, accidents, or injuries.
- Entries should include: operator's name or initials, lab in which the individual works, type and duration of load, date, and time.
- The log sheets must be kept adjacent to the autoclave.
- An example of the log sheets utilized are provided at the end of this document.

G. Maintenance and Repair

- No person shall operate the autoclave unless the autoclave is in good repair.
- Only qualified professionals are permitted to make repairs.
- Report possible malfunctions to the individual responsible for the autoclaves, noted on the front page of this document.

H. Autoclave Validation

- Autoclaves shall be validated to ensure sterilization utilizing biological indicator assays. Validations
    - **Time tape or steam indicators DO NOT validate sterilization**
    - Typical biological indicator assays involve sterilization or inactivation of spores which demonstrate sterilization rather than just heat.
        - An Attest™ Biological Indicator assay by 3M or equivalent is recommended.
    - Validation utilizing a biological indicator assay should be conducted by inserting the biological indicator into a run to be validated.
    - It is critical that the validation run is similar to a regular run, both in terms of sterilization time, but also load conditions
        - For example: if an autoclave is regularly utilized for a liquid run of 10L of liquid, 10L of liquid need to be validated with the biological indicator placed in one of the liquid containers
        - All individual labs are encouraged to conduct their own validation on their lab-specific loads
- A log of autoclave validation should be kept on or near the autoclave.
    - Frequency:
        - Users running loads 2 times or less per month: Autoclaves must be validated every six (6) months at a minimum.
        - Users running loads 1 or more times per week: Autoclaves must be validated every month at a minimum.
        - A validation should be run anytime repair or maintenance has occurred on an autoclave.
- An example of the validation log sheets utilized are provided at the end of this document.

IV. Contingency Plans

A. Equipment Malfunction

- If the autoclave does not operated exactly as expected, do not attempt to fix the problem. A notice shall be placed on the autoclave indicating that it is not to be used until the problem is diagnosed and corrected.
- Record the problem in the autoclave use log.
- Contact the individual responsible for the autoclave and your supervisor or PI to report the problem.
- Only qualified professionals are permitted to make repairs.

B. Incident Response

- All incidents, including a spill or release of biohazardous materials or recombinant DNA must be reported to your supervisor and to the University of Wyoming Safety Office at 766-3277.
- If any injury occurs:
    - Seek first aid
    - If necessary, seek medical assistance at Ivinson Urgent Care or Ivinson Memorial Hospital Emergency Department or by dialing 911 if the injury is serious.
- If clothing is soaked in hot water/steam, remove the clothing and place the injury in cool water.
- Place a notice 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 by the responsible person for the autoclave.

C. Spill Clean-up

- Spills may occur from a boil-over or breakage of containers.
- No operation of the autoclave is allowed until the spill is cleaned up.
- The operator is responsible for clean-up of spills.
    - Wait until the autoclave and materials have cooled to room temperature before attempting clean-up.
    - Contain the spilled material using paper towels.
    - Use the laboratory's spill kit if necessary.
- Review the MSDS, if appropriate, to determine appropriate PPE, spill cleanup, and disposal protocols that are necessary.
- Dispose of the waste following the protocol appropriate for the material.
- Cracked glassware must be disposed of properly.
    - Wear gloves and use dust pan or tongs to pick up broken glass.
    - Dispose in broken glass container.
- Record the spill and clean-up procedure in the autoclave use log.

References

- Association for the Advancement of Medical Instrumentation. (2017). *ANSI/AAMI ST79:2017/(R)2022 comprehensive guide to steam sterilization and sterility assurance in health care facilities.*
- Centers for Disease Control and Prevention, & National Institutes of Health. (2025). *Biosafety in microbiological and biomedical laboratories (6th ed.)*. U.S. Department of Health and Human Services.

Reviewed by the University of Wyoming Safety Office, Biosafety Officer, and the Institutional Biosafety Committee in October 2026.

Autoclave Usage Log

Name	Lab	Cycle	Date	Time

Comments:

Revised and reviewed 10-2026

Validation Log for ____ year

Month	Description of run	Date/Time/ Lab	BI Control (Circle)	BI Test (Circle)
January	Lab PPE waste and Agar Plates	01/01 @ 12:00 pm XYZ Lab	Growth-Pass No Growth- Fail	Growth- Fail No Growth- Pass
			Growth-Pass No Growth- Fail	Growth- Fail No Growth- Pass
			Growth-Pass No Growth- Fail	Growth- Fail No Growth- Pass
			Growth-Pass No Growth- Fail	Growth- Fail No Growth- Pass
			Growth-Pass No Growth- Fail	Growth- Fail No Growth- Pass
			Growth-Pass No Growth- Fail	Growth- Fail No Growth- Pass
			Growth-Pass No Growth- Fail	Growth- Fail No Growth- Pass
			Growth-Pass No Growth- Fail	Growth- Fail No Growth- Pass
			Growth-Pass No Growth- Fail	Growth- Fail No Growth- Pass
			Growth-Pass No Growth- Fail	Growth- Fail No Growth- Pass
			Growth-Pass No Growth- Fail	Growth- Fail No Growth- Pass
			Growth-Pass No Growth- Fail	Growth- Fail No Growth- Pass
			Growth-Pass No Growth- Fail	Growth- Fail No Growth- Pass
			Growth-Pass No Growth- Fail	Growth- Fail No Growth- Pass
			Growth-Pass No Growth- Fail	Growth- Fail No Growth- Pass
			Growth-Pass No Growth- Fail	Growth- Fail No Growth- Pass
			Growth-Pass No Growth- Fail	Growth- Fail No Growth- Pass
			Growth-Pass No Growth- Fail	Growth- Fail No Growth- Pass

Comments:

Autoclave Training / Standard Operating Procedure Log

I certify that I have been trained for autoclave usage and have read & understand the autoclave standard operating procedure (SOP).

Print Name _____ Signature _____ Date _____

Working in the following lab(s) _____

Responsible individual who trained the above for autoclave usage.

Print Name _____ Signature _____ Date _____

I certify that I have been trained for autoclave usage and have read & understand the autoclave standard operating procedure (SOP).

Print Name _____ Signature _____ Date _____

Working in the following lab(s) _____

Responsible individual who trained the above for autoclave usage.

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I certify that I have been trained for autoclave usage and have read & understand the autoclave standard operating procedure (SOP).

Print Name _____ Signature _____ Date _____

Working in the following lab(s) _____

Responsible individual who trained the above for autoclave usage.

Print Name _____ Signature _____ Date _____

Revised and reviewed 10-2026