ARIZONA STATE UNIVERSITY

Department of Environmental Health & Safety
Biosafety & Biosecurity

Autoclave Safety Manual

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I. Preface

The Arizona State University (ASU) Autoclave Safety Manual is a resource for information, guidelines, policies, and procedures enabling and encouraging staff using autoclaves to work safely and eliminate, or reduce, the potential for exposure to hazards or potentially infectious materials. It is expected that the Principal Investigator (PI) and supervisory personnel will supplement this safety manual with hands-on training on the specific autoclave to be used. Hands-on training should include practices and procedures specific to the autoclave and the unique activities taking place in the laboratory.

All biological waste at ASU must be decontaminated prior to disposal or transferred to an approved waste hauler. All biological waste must be disposed in a manner that protects students, employees, contractors, community, and the environment from biological hazards. Procedures for decontamination are based on state and federal law, requirements from the Occupational Safety and Health Administration (OSHA), Centers for Disease Control and Prevention (CDC), and National Institutes of Health (NIH). Decontamination can be achieved with chemical disinfection or thermal sterilization (e.g., steam autoclaves).

This manual is designed to provide guidance for the safe use of autoclaves at ASU. The procedures described in this manual comply with local, state, and federal regulations and ASU policies.

If you have any questions regarding this manual or autoclaves at ASU, please contact ASU Biosafety and Biosecurity at (480) 965-5389 or email biosafety@asu.edu.

This manual will be reviewed, and revised as necessary, at least annually.

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II. Overview

Autoclaving is a process used to destroy microorganisms and decontaminate biohazardous waste and microbiological equipment used at Biological Safety Levels (BSL) 1, 2, 3 and 4 (see the ASU Biosafety Manual for more information regarding Biological Safety Levels). The autoclave is a steam sterilizer comprised of an insulated chamber where saturated steam is used during timed cycles to raise both temperature and pressure to perform sterilization.

For proper sterilization, a specified temperature, ranging from about 121°C (or 250°F) to 132°C (or 270°F) and pressure, ranging from about 15 to 30 pounds per square inch (psi), is maintained throughout one or more cycles for a specified amount of time. This varies depending on the type and amount of materials being autoclaved. Most steam autoclaves use 121°C at 15 psi for 30 minutes, for example.

Figure 1. Diagram of an Autoclave

The potential safety risks for autoclave operators are related to the high temperatures and pressures being created by the unit. These risks include:

- Burns from touching scalding equipment and materials (e.g., internal autoclave surfaces, chamber walls, and door).
- Steam burns from residual steam coming out from the autoclave and materials at the end of the run.
- Hot fluid scalds from boiling liquids and spillage in the autoclave.
- Hand and arm injuries when opening and closing the door.
- Bodily injury if there is an explosion due to incompatible materials being autoclaved.
- Harm from inhalation or other exposure to fumes and vapors from chemicals that should not have been autoclaved (e.g., bleach or other hazardous chemicals).
- Biological hazards are possible from handling certain biological materials before autoclaving or if they were not autoclaved properly (e.g., autoclave did not reach sterilization temperature during the run).

III. Training

Training is required before operating autoclaves and includes the following:

- Read and understand the material presented in this Autoclave Safety Manual.
- Read and understand the ASU Autoclave Standard Operating Procedure (SOP).
- Complete the ASU Autoclave Safety Training (available on Blackboard).

  Arizona State University's Environmental Health and Safety (EH&S) Biosafety and Biosecurity Team is the recipient of a prestigious national health and safety award for developing this autoclave training video. In July 2016, the project won first place and was granted the Innovation Award by the Campus Safety, Health, and Environmental Management Association (CSHEMA), a professional organization honoring the achievements of higher education institutions by offering awards recognizing outstanding programs and innovations on campus since 1954.

- Participate in individualized, hands-on training tailored for each laboratory. Principal investigators, lab managers, or immediate supervisors must provide this training to individuals prior to allowing them to work with and autoclave. The training should be conducted for each autoclave the individual will be using.

Additional autoclave resources:

- ASU Biosafety Manual
- CDC/NIH Biosafety in Microbiological and Biomedical Laboratories

IV. Preparation

A. Personal Protective Equipment

Personal Protective Equipment (PPE) is required at all times when working in laboratories and areas where autoclaves are operated. Before entering laboratories and autoclave areas, ensure personnel are wearing the following:

- Closed-toed shoes
- Long pants
- Gloves
- Lab coat
- Eye protection

It is important that the PPE fits correctly to cover as much skin as possible. When unloading the autoclave, heat-resistant gloves must also be used; a heat-resistant apron is recommended.

Additional PPE may also be used to safety load and unload the autoclave. Be sure to identify the location of additional PPE such as heat-resistant gloves, tongs, rack tools, and other safety equipment.

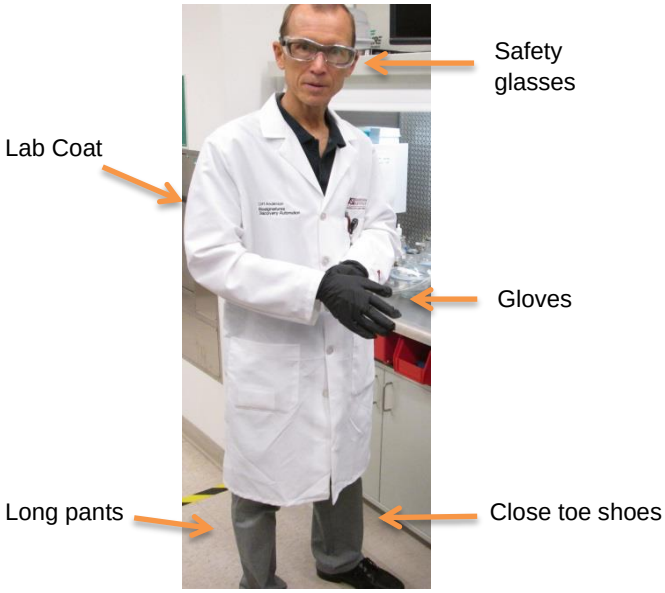

Figure 2. Personal Protective Equipment

Figure 3. Heat-Resistant Gloves
(Source: http://www.sigmaaldrich.com)

B. Secondary Containers

All materials to be autoclaved should be placed into an autoclave-resistant secondary container (such as a stainless steel or polypropylene pan or tray) to secure items and contain possible spills. Ensure the secondary container is large enough to contain a total spill of the liquid contents.

Figure 4. Secondary Containers

C. Unsuitable for Autoclaving

Always ensure material is safe for autoclaving:

- NEVER AUTOCLAVE FLAMMABLE, REACTIVE, CORROSIVE, TOXIC, or RADIOACTIVE MATERIALS even if mixed with biological waste.
- Do not autoclave bleach or any liquids containing bleach.
- Do not autoclave mixed waste materials.
- Do not autoclave liquids in sealed containers.
- Do not autoclave resin plastics that will melt (e.g., HDPE, LDPE, PET or PETG).
- Do not autoclave items containing polystyrene (PS), polyvinyl chloride (PVC), nylon, acrylic, or polyurethane tubing.
- Do not autoclave items that touch the sides of the interior surfaces of the autoclave chamber.

D. Preparing and Packaging Materials

The following considerations should be followed when preparing and packaging materials for the autoclave:

- Glassware must be heat-resistant (such as Pyrex or Type I borosilicate) and be free of cracks, scratches, or other damage. Always inspect glassware prior to autoclaving.
- Wash and rinse glassware with distilled water before autoclaving and loosen caps and stoppers. Always place upright in the autoclave pan.

Figure 5. Pyrex Glassware
(Source: www.pyrex.com)

- For dirty glassware, add detergent and water up to two-thirds (2/3) of the container, cover with tin foil or loose cap, and place directly in a heat-resistant pan with one inch of distilled water.

Figure 6. Autoclavable Liquid Containers

- For liquids, loosen all lids to prevent pressure buildup. All containers must be covered with a loosened lid, aluminum foil, or steam-penetrating stopper.

- Liquid containers must be made of Type 1 borosilicate glass and contents must not exceed two-thirds (2/3) volume.

- Place liquid containers upright inside an autoclave pan and add one inch of distilled water to the bottom of the pan.

Figure 7. Autoclave Pouches (Source: www.doctor-mayer.com)

- Loose, dry materials must be wrapped in steam-penetrating paper, contained in plastic autoclave bags or pouches, or loosely covered with aluminum foil. Wrapping items too tightly will impede steam penetration thereby decreasing the effectiveness of the process.

- Dry materials should be placed flat in the autoclave pan. Allow sufficient space between items for steam to penetrate.

- Test tubes or Erlenmeyer flasks should be placed in a metal rack and covered with aluminum foil.

Figure 8. Placement of Test Tubes and Erlenmeyer flasks

- All plastics placed into the autoclave must be able to withstand the extreme conditions inside the unit. Examples of plastic that may be used include:
  - Polypropylene (PP) and polypropylene copolymer (PPCO)
  - Polycarbonate (PC) (may only withstand 30 to 50 cycles)
  - Fluoropolymers: Teflon PFA, FEP, or ETFE
  - Polymethylpentene (PMP)
  - Polymethyl methacrylate (PMMA)

- Sharps waste must be contained in a sealed sharps container.
- When autoclaving solid biohazardous wastes, ensure that a red, orange or clear polypropylene autoclave bag clearly labeled with biohazard symbol or the word “biohazard” is used. Also, follow these steps when autoclaving bags of waste:
    - Do not over pack or overfill the bag more than two-thirds (2/3) full. The items should be loose within the bag to allow the steam to flow.
    - Add 250 mL of distilled water to the bag to ensure enough steam will circulate inside the bag.
    - Tie the bag loosely with tape or a twist tie to allow steam to escape.
    - Avoid puncturing bags when filling with waste materials. If the bag becomes punctured or ripped, use a new one or place into another (double bag).
    - Place bag into a heat-resistant secondary container. The bag should be able to fit properly in the secondary container within the autoclave chamber.
- Include a process indicator (e.g., autoclave tape) on load items (see Section V – Quality Assurance and Monitoring).

Figure 9. Biohazard Bag

V. Quality Assurance and Monitoring

All items that are autoclaved must have a process indicator present to verify that sterilization was achieved. To ensure quality assurance, the use of a biological monitoring system is highly recommended.

A. Autoclave Tape

Autoclave tape is a type of process indicator made from adhesive tape. It looks similar to masking tape except it is embossed with indicator lines. The purpose of autoclave tape is to indicate whether a specific temperature has been reached. One limitation of autoclave tape is it only measures the temperature and cannot measure all of the sterilization conditions such as length of cycle(s) and pressure.

Figure 10. Autoclave Tape

Autoclave tape works by changing color after exposure to temperatures commonly used in sterilization processes (typically 121°C in a steam autoclave). When the cycle reaches the appropriate temperature, the indicator lines turn from a light color to a dark color (see Figure 10). Check the process indicator at the end of each run to ensure steam processing was successful. Repeat autoclave run if indicator has not changed colors.

B. Biological Monitoring Systems

Commercially available biological indicators (bioindicators) should be used as part of an effective biological monitoring system. Bioindicators include the use of a dried or liquid suspension of nonpathogenic spores (e.g., *Bacillus stearothermophilus)*.

Figure 11. Examples of Bioindicators
(Source: www.microqa.com)

Dried spores may be embedded in strips and liquid suspensions may be contained in glass ampules. Dried spore strips may require a separate growth medium contained in a glass ampule. Most bioindicators require an incubation period of 24 to 48 hours at 50 to 60°C after the autoclave run. An autoclave should not be used until biological indicators demonstrate an effective sterilization validation.

C. Validation Testing

Validation testing using a bioindicator is recommended on a quarterly basis (i.e., every three months). A validation test should also be performed when there are the following changes in laboratory operations:

- Different types of wastes are being autoclaved.
- Packaged materials (such as autoclave bags or pouches) are being used.
- The load configuration, weight, or volume increases or decreases.
- Cycle parameters are altered.
- Small or routine repairs or calibrations have been made to the autoclave.

A validation is also required when:

- As part of initial set up and installation of a new autoclave or an autoclave returning to service from storage.
- After an autoclave has been moved.
- After a malfunction is suspected.
- After a major repair has been conducted.

A suggested testing protocol is provided in Appendix A.

A list of shared autoclaves and locations is provided in Appendix B.

VI. Safety Inspections

Conduct a safety inspection before and after each autoclave run. A safety inspection includes:

- **Inspecting visible door gaskets and seals for wear and tea**r. There should be no cracks, tears, folding, or shredding and all gaskets and seals should look smooth and pliable.

- **Check the drain screen and hoses**. If the drain screen is blocked with debris, a layer of air may form and prevent proper operation. Clean out any debris with caution as to not damage the screen. If the drain hose is not attached properly and positioned correctly, spills and flooding may occur.

- **Review the Autoclave Use Log for any notes on issues or malfunctions.** If issues or malfunctions are suspected or occurring, report these to the person responsible for the autoclave and your supervisor. Do not use the autoclave until it is cleared for use.

VII. Loading an Autoclave

Follow these steps when loading an autoclave:

1. Wear appropriate PPE including, including closed-toed shoes, long pants, gloves, lab coat, and eye protection. Heat-resistant gloves should also be used. Wear an apron and face shield when handling hot liquids.

Figure 12. Heat-Resistant gloves

2. Always use a secondary container. Do not mix incompatible materials.

3. Do not overload. Leave sufficient room for steam circulation. If necessary, place containers on their sides to maximize steam penetration and avoid entrapment of air.

4. Place process indicators (e.g., autoclave tape) on several items in the load (see Section V – Quality Assurance and Monitoring).

5. Close the door and latch it properly. Depending on the autoclave, you may have to manually close and secure the door without over-tightening it. Newer autoclaves seal automatically.

6. Human error plays a role in inadequate sterilization. Be sure to check the following:

   - Items are adequately clean to ensure steam penetration.
   - Packaged materials are not too large or dense for cycle parameters.
   - Loading techniques allow free-flowing air and steam penetration.
   - Correct autoclave parameters are set.
   - The run has started and the entire load is not inadvertently left unprocessed.

VIII. Operation

Prior to using an autoclave, always consult with the lab supervisor and equipment manual to choose the appropriate cycle for the material (e.g., gravity, liquid, or dry). The ASU Autoclave Poster offers cycle suggestions. The sterilization temperature should be set to 121°C (250°F) or higher, depending on the materials to be autoclaved.

When autoclaving liquids, consider the use of these cycle times to ensure proper sterilization:

- **Less than 500 milliliters (mL):** 30 minutes
- **More than 500 mL but less than 2 liters (L):** 45 minutes
- **More than 2 L:** 1 hour

When autoclaving solid or mostly solid biohazardous waste, consider the following cycle times:

- **Glassware and pipette tips:** 30 minutes
- **Biohazardous waste:** 45 minutes or longer

After beginning the run, complete the Autoclave User Log (see Appendix C for an example log). Include your full name and contact phone number, the date, time, type of material, cycle parameters, and any comments including tests, maintenance, or malfunctions.

Check the autoclave 20 minutes into the run to ensure that the target temperature has been reached (minimum temperature of 121^0C (250^0F). Check the chamber and jacket pressure gauge for minimum pressure of 15 pounds per square inch (psi). Do not attempt to open the door while the autoclave is in operation. A complete run usually takes about one hour to one hour and a half.

If problems with the autoclave are observed or perceived, abort the run immediately and report it to your supervisor and the person in charge of the autoclave.

IX. Unloading an Autoclave

Follow these steps when unloading an autoclave:

Figure 13. Unloading an Autoclave

1. Wear appropriate PPE including, including closed-toed shoes, long pants, gloves, lab coat, and eye protection. Heat-resistant gloves should also be used. Wear an apron and face shield when handling hot liquids.

2. Ensure that the run has completed and autoclave readouts (e.g., screen, printer paper) indicate that both temperature and pressure have returned to a safe range before opening the door.

3. Allow the autoclaved load to stand for 10 minutes before removal. This will allow for any steam to clear and trapped air to escape from hot liquids, reducing risk to operator.

4. Opening the door:

   To unload an autoclave with a manual door:

   - Position the body and head as far away from the door as possible.

   - Cautiously unlock the door and slowly open it to no more than one inch to allow for residual steam to release and the pressure within liquids and containers to equalize.

   - Allow a few minutes for the steam to be released before completely opening the door and unloading contents.

   To unload an autoclave with an automatic door:

   - Stand at a safe distance.

   - Push the open button.

   - Stand back as steam is being released. Allow a few minutes for steam to be completely released before unloading contents.

5. Use extreme caution not to agitate containers containing superheated liquids or remove caps before unloading.

6. Check the process indicator (e.g., autoclave tape) to ensure a successful run.

7. Cautiously remove items from the autoclave and place them in a clearly marked area that indicates the items are “hot.”

8. Do not remove the items from the “hot” area until they have cooled down to room temperature.

9. Allow autoclaved materials to cool to room temperature before transporting. **Never transport superheated materials.**

10. Close the autoclave door.

11. Perform a safety inspection and record any issues or malfunctions on the log.

12. Report issues or malfunctions to your supervisor and the person in charge of the autoclave.

X. Waste Disposal

Sterilized biohazard bags and sharps containers should be placed in the designated, large biological waste collection bins available inside the autoclave room. Do not overfill the collection bins. Once they are two-third (2/3) full (or contain two or three large bags) contact EH&S Waste Management for pick up. An online ticket system is available for all waste.

Please refer to the ASU Fact Sheet for Biohazardous Waste Handling Procedures for more information.

XI. Autoclave Maintenance and Communication

Communication is critical to ensure that equipment continues to be safe and operational. Follow these steps to ensure a safe work environment:

- **Read and understand the manufacturer's equipment operation manual.** Become familiar with functionality, required routine maintenance, and troubleshooting.

- **Ensure that a Maintenance Log Book is completed.** This log should be provided by the autoclave manufacturer. Keeping a chronological maintenance log book ensures all routine maintenance, as well as malfunctions and repair outcomes, are available for review. Document your name, date, and contact information on each entry. Add the repair technician's contact information if available for use if needed for future repairs.

- **Report all malfunctions to appropriate personnel.** Communicate with your supervisor and the person in charge of the autoclave regarding the status of the autoclave if any issues or malfunctions occur. Document a summary of these types of communications in the Maintenance Log Book.

- **Never use a malfunctioning autoclave.** It is important NOT to operate an autoclave if it is not in proper working condition as this could make a problem worse or present a safety hazard.

- **Do not attempt to repair an autoclave.** Only qualified professionals are permitted to make repairs. Do not attempt to fix, adjust, or repair anything that is not considered a user function or routine maintenance per the equipment manual. Report the autoclave malfunction to your supervisor and person in charge of the autoclave and wait for clearance before operating the unit.

- **Place an out of service sign on malfunctioning autoclaves.** Affix an "Out of Service" sign on any malfunctioning unit to prevent other individuals from using the autoclave. Include the date and time unit was placed out of service, a brief description of the issue, and your name and contact information. Include an estimated repair date if available.

XII. Spill Cleanups

Spills may occur from a boil-over, spilled liquid containers, broken or cracked containers, or a blocked drain. Follow these steps in the event of a spill and to ensure the autoclave is placed out of service until the spill is cleaned up.

1. Wait until the autoclave and materials have cooled to room temperature before attempting cleanup.

2. Review the manufacturer's instructions and Safety Data Sheet (SDS) to determine appropriate PPE for spill cleanup and disposal protocols.

3. Contain spilled material with paper towels or other absorbent material. Use your laboratory spill kit if necessary.

4. Dispose of waste in accordance with regulatory requirements.

5. If materials have been mixed, follow the cleanup and disposal protocol for the most hazardous component.

6. Cracked or broken glassware must be disposed of properly.

7. Record the spill and cleanup procedure on the Autoclave Use Log (see Appendix C).

8. If the autoclave requires repairs, notify your supervisor and the person in charge of the autoclave.

Appendix A – Suggested Validation Testing Protocol

1. Place the bioindicator in the center of a typical autoclave load. If validating a liquid cycle, consider placing it inside an extra container filled with approximately the same volume of distilled water as the items to be autoclaved.

2. Place autoclave tape on several items in the load (e.g., bottles, tubes, flasks, pipette tip boxes).

3. Process the load as usual, selecting typical cycles and times.

4. Open the door for a minimum of 10 minutes prior to removing the bioindicator to allow for a brief cooling period. ***Warning:*** *Crushing or excessive handling of the bioindicator before cooling may cause the glass ampule to burst which may result in personal injury from flying debris. Use safety glasses and appropriate gloves (heat-resistant and puncture-resistant) when removing or intentionally crushing bioindicator.*

5. Incubate the bioindicator within two hours or as recommended by the manufacturer. Use a negative control during incubation which can be a fresh bioindicator from storage.

6. Examine the bioindicator at regular intervals during incubation (e.g., 8, 12, 18, 24 and 48 hours) for any color change. For example, most bioindicators will change color from pink to yellow (a positive read). Yellow usually represents bacterial growth whereas no color change (pink) indicates a negative read and adequate sterilization process.

7. Observe and document results of the run to determine if the sterilization process parameters successfully produced negative bioindicators and chemical indicators with complete end point color changes.

8. Record data on a separate test log including bioindicator lot number and expiration date, incubation conditions, observations and results. Make an entry onto the Autoclave Use Log (see Appendix C) for each run completed and indicate test details in the comments section.

9. Consider photographing the bioindicators and printing or saving the images as data.

10. Notify your supervisor and person in charge of the autoclave of any positive test results as soon as the first evidence of growth is noted. For example, growth may indicate that a longer cycle time is required to achieve sterilization.

If changes are made to the autoclave cycles, always retest the sterilization process to confirm.

Appendix B – Common Use Autoclave Locations

- **Tempe Campus**
  - Biodesign: - A295
    - A395
    - B169
    - B369
  - ISTB4: - 331 B1
    - 431 A2
  - ISTB1: - 158
    - 258
    - 358
    - 458
  - Life Sciences: - LSA L1-26
    - LSC 588
    - LSE S62
    - LSE B62
    - LSE B64
    - LSE 345
    - LSE 441
    - LSE 537
    - LSE 627
    - LSE 735
    - LSE 905E
  - PSD: - 228
- **Downtown Campus**
  - UCENT: - 360D
- **Polytechnic Campus**
  - SANTAN: - 208
    - 308
- **West Campus**
  - CLCC: - 338
    - 357

Appendix C - Autoclave Use Log

Printed Name *(Do not use initials)*	Contact Phone	Date	Time	Type of Materials	Cycle Parameters	Comments