I. PURPOSE:

The purpose of this standard operating procedure is to outline the safe operation and maintenance of autoclaves and sterilizers in laboratories at the UT Health Science Center at San Antonio.

There are several types of sterilizers (steam autoclaves, Ethylene Oxide gas, plasma, etc.) used in the decontamination and sterilization process in research and healthcare. For the purposes of this SOP, only the procedures for steam sterilizers (also known as autoclaves) will be discussed.

Despite built-in safeguards, an autoclave presents the possibility of serious injury to individuals from hot surfaces and from the release of steam. It is important, therefore, that individuals understand the proper operation, limitations, and safeguards for sterilization by autoclaving.

II. SCOPE

This standard operating procedure (SOP) applies to all individuals (employees, students, visiting scientists, and volunteers) at UTHSCSA who operate and maintain autoclaves. This SOP should be used in conjunction with the autoclave manufacturer’s manual and other required departmental/clinical procedures.

III. RESPONSIBILITIES

1. Principal Investigator/Department.
   The Principal Investigator and department has overall responsibility for ensuring employees, students and volunteers under their supervision:
   a. Adhere to all guidelines, policies and regulations pertaining to the use of autoclaves.
   b. Complete all appropriate safety training and document the training.
   c. Are made aware of and trained in the hazards associated with operation of an autoclave.
d. Use the appropriate personal protective equipment (PPE) as necessary.
e. Report hazardous material spills to Environmental Health & Safety.
f. Maintain the autoclave as directed by manufacturer’s specifications.

2. Users (Employees, Students, Visiting Scientists and Volunteers).
It is the responsibility of each individual to:

a. Comply with all guidelines outlined in this standard operating procedure.
b. Attend all appropriate safety training related to autoclave operation.
c. Notify their immediate supervisor of any problems with an autoclave.
d. Notify their immediate supervisor or any injuries related to its use.
e. Inform their immediate supervisor or EH&S of any unsafe practices or conditions.

IV. BACKGROUND and DEFINITIONS

1. BACKGROUND
Sterilization refers to the complete killing of all living organisms, including spores. Common sterilization techniques include the application of wet heat, dry heat, chemicals, and radiation. The type of material, the container, and quantity of items to be sterilized determines which method to use. Various types of equipment are used for sterilization in the research and clinical laboratory and laboratory animal facilities, but the autoclave is the primary means of sterilizing supplies. The autoclave is also used to sterilize infectious waste. Sterilization will occur when the conditions of steam, pressure, temperature, and time are met.

2. ASSOCIATED RISKS
Autoclaves are sterilizers using high pressure and high temperature steam to kill microorganisms and render biohazardous material inactive.
The potential safety risks for the operators are:
- Heat burns – from hot materials and autoclave chamber walls and door
- Steam burns – from residual steam coming out from the autoclave and materials on completion of the cycle
- Hot fluid scalds – from boiling liquids and spillage in autoclave
- Hand and arm injuries when opening and closing the door
- Bodily injury if there is an explosion

V. PERSONAL PROTECTIVE EQUIPMENT (PPE) AND AUTOCLAVE SAFETY

1. Personal Protective Equipment:
Individuals are required to wear the following PPE when a cycle is complete and removing items from the autoclave.
- Heat-insulating gloves that provide complete coverage of hands and forearms
• Face shield that provides complete coverage of the face and neck when hot liquids are handled
• Splash or rubber apron that provides complete coverage of the chest and legs – a lab coat is sufficient if no hot liquids are handled
• Long pants or long skirt and closed-toe & slip resistant footwear

2. Autoclave Safety:

• Never seal containers; under pressure they pose an explosion risk.
• Never open the door to the autoclave if there is water running out the bottom. Clogged steam lines, equipment malfunction, or plugged drains may cause a buildup of scalding water.
• Wait for the pressure to reach zero and the temperature is at or below 121°C before opening the door at the end of a cycle to avoid steam burns and shattered glassware. Do not stand directly in front of the door.
• Never remove superheated liquids. Superheating is a condition that occurs when liquids are at a temperature above their normal boiling point but do not appear to be boiling. Any disturbance of the liquid could cause some of it to violently flash to steam and spraying. In situations where personnel are in a hurry to remove flasks or bottles from the autoclave, the superheated liquids may boil out of their containers or explode.

Never autoclave the following:

• Hazardous chemicals (including items contaminated by hazardous chemicals). Do not autoclave flammable, reactive, corrosive, toxic chemicals (e.g., alcohols, chloroform, acetic acid, formalin, or fixed tissues), oils, and samples containing solvents or substances that may emit toxic fumes. **Bleach-containing waste cannot be autoclaved.**
• Lab coats that have been contaminated with chemicals should not be autoclaved but cleaned by an approved laundry service or disposed of as chemical waste.
• Dried bleach and bleach-associated materials, or nitrocellulose; both compounds pose a fire or explosion risk.
• Radioactive materials: Contact the Radiation Safety Program for information on proper disposal of radioactive materials.
VI. MATERIAL PREPARATION

- Ensure that the material is able to be autoclaved.
- Prepare and package material suitably.
  - Loose dry materials should be wrapped or bagged in steam-penetrable paper or loosely covered with aluminum foil. Wrapping too tightly will impede steam penetration, decreasing efficiency of the process.
  - All containers should be covered by a loosened lid or steam-penetrable stopper.
  - Inspect the glass to make sure there are no cracks.
  - Containers/bottles of liquid should be a maximum of 2/3rds filled, with caps loosened to allow for pressure changes or it may explode. Keep 1-2 inches of space between the bottles.
  - Glassware should be heat-resistant borosilicate.
  - Plastics should be heat-resistant eg: polycarbonate (PC), PTFE ("Teflon") and most polypropylene (PP) items.
  - Sharps must be in a designated ‘Sharps’ container or tray.
  - Items or baskets should be tagged with autoclave tape.
  - Loosen all lids to prevent pressure buildup.
- Add water to containers/autoclave tray as appropriate.
- Place items in containers to secure and contain spills:
  - Items should be placed in a stainless steel or autoclavable plastic container/pan for their stability and ease of handling.
  - The pan must be large enough to contain a total spill of the contents.
  - Bags must not be tightly sealed as steam cannot penetrate tightly sealed bags.
- Small items to be autoclaved should be bagged or wrapped in heat-resistant plastic, paper, or cloth.
- Sterilized supplies must be labeled with date autoclaved/processed. Date of expiration is determined using the following criteria:
  - Sterile packs/items should be stored in clean, dry enclosed cabinets and drawers free from moisture and dust.
  - Sterile packs prepared by research or technical staff, when stored in enclosed cabinets/drawers in a dust-free, insect-free, and well-ventilated area, can be considered sterile up to 1 year providing the packaging remains dry and intact, depending on manufacturer’s recommendations. Sterile packs stored on open shelves may have a reduced shelf life.
  - The manufacturer generally stamps commercially prepared sterilized packs with an expiration date.
  - A shelf life of 1 year is suggested for commercially prepared packs without an expiration date, providing the pack is stored in an enclosed cabinet/drawer, remains dry, and packaging is intact. Label with the date of receipt.
The quality of the packaging material, storage and transport conditions, and the amount of handling all contribute to maintaining sterility of the package and its contents.

Any package that is wet, torn, dropped on the floor, damaged in any way, or past its date of expiration shall not be used. When such events occur, the contents should be removed, repackaged, and re-sterilized.

- Autoclave steam indicator tape should be used in each autoclave load. It may be used to secure packages and also to indicate that those packages have been exposed to the proper levels of heat and moisture needed to achieve sterility. After proper exposure, dark diagonal lines appear on the tape. While this color change is not a guarantee of sterility and should be evaluated with biological monitoring tests, it does indicate that all factors necessary for sterilization were present during processing.

- Drinking water (for animals) is autoclaved by placing filled water bottles in wire baskets and covering the wire basket with a suitable cover (e.g., tyvek, or similar material) prior to autoclaving. Autoclaved water bottles remain covered from time of autoclaving until time of use.

- When autoclaving biohazardous waste:
  - In the Biosafety cabinet, infectious waste should be discarded into an autoclavable biohazard bag or in containment vessels.
  - Bags/containers should not be overfilled, half to three quarters full is recommended.
  - The autoclave bag is loosely tied closed. Spray bag off with disinfectant prior to removing from the cabinet.
  - Biohazard bags/containers should be labeled with an autoclave indicator so that it can be readily determined that the container has been autoclaved.
  - These materials should be processed as soon as possible.

VII. BASIC AUTOCLAVE OPERATION

The following are basic instructions for autoclave use but do not replace the manufacturer's operating instructions and hands-on training. Before using any autoclave for the first time, read and thoroughly understand the owner's manual because many makes and models have unique characteristics.

LOADING

1. Make sure there is paper in the recorder.
2. Observe display to ensure it reads “Door Unlocked V=0 in Hg.”
3. For bench top units that do not have inline steam, check and fill the reservoir with deionized water to the fill line (see manufacturer’s instructions).
4. Load items into the autoclave chamber per manufacturer’s specifications.
5. Items to be sterilized are loaded into the autoclave in a manner so that nothing touches the inside of the chamber. Items should be placed into an autoclavable tray on a shelf or rack and never placed directly on the autoclave chamber bottom or floor. DO NOT OVERLOAD. Leave sufficient room for steam circulation.
6. Items to be sterilized should be placed into the autoclave so that steam can uniformly flow between items and so that no air pockets are formed between or around them.
7. Check the drain screen to make sure that it is not plugged or obstructed.
8. As necessary, insert Spore Strips (see Sterilization Assurance).
9. Close door and turn wheel clockwise until display changes from “Door Unlocked” to “Time:” then tighten down wheel a minimum of two additional turns.

10. Choose the desired cycle for the type of load, gravity or liquids (e.g. liquids, dry, etc.) and set the appropriate time and temperature. For many departmental autoclaves, the cycle times and temperature are preset (select cycle 1 (15 minutes), 2 (30 minutes), etc.).
11. The sterilization time will vary according to the contents and how the load is packaged and should be measured after the temperature of the materials reaches 121°C and 15 pounds per square inch (PSI). It can be variable with a minimum of 15 minutes.
12. The sterilization temperature. Unless specifically instructed, the chamber temperature is set to 121°C (250°F).
13. A dry cycle may be desired.
14. Before commencing a liquid cycle, ensure the chamber pressure is ‘0’
15. Check the desired parameters and then press cycle “ON”.

CAUTION: If any problems (safety, wrong cycle) are noted during the cycle, depress the “RESET” button. The cycle can not be restarted until the pressure reaches “0 psiG” and the “Complete V = 0 inHg” message is observed.
16. Fill out the autoclave log sheet if this is required by your department.

UNLOADING

1. When cycle is complete, an audible signal will usually sound in conjunction with a light or message indicating that the cycle is complete. Do not attempt to open the door while the autoclave is operating. Observe display to ensure the pressure reads “Complete V=0 in Hg.”

2. Turn door wheel counterclockwise until display reads “Door Unlocked.” Slowly open the door and only slightly for about 10 minutes to allow steam to escape. Keep your head, face and hands away from the opening.

3. After 10 minutes, open the door completely and allow the materials inside the autoclave to cool for at least 10 minutes before unloading the autoclave.

4. Observe the autoclave tape for color change (turns black) and the printer tape from the recorder to ensure that the time and temperature (reached 250°F/121°C) were attained for the cycle. NOTE: If the autoclave tape did not change color or the temperature did not read 250°F/121°C or higher for the appropriate amount of time, contact supervisor for further instructions. The load will need to be re-autoclaved in another autoclave. Place a sign on the autoclave “DO NOT USE”.

5. If there are sharps or broken glass, use tongs or cut resistant gloves to remove these materials from the autoclave.

VIII. STERILIZATION ASSURANCE (VERIFICATION)

Sterilization procedures should be monitored through a combination of mechanical, chemical, and biological techniques designed to evaluate the sterilizing conditions and the procedure’s effectiveness.

- **Printer Tape** – read the tape to ensure the temperature reached 250°F/121°C and 15 pounds per square inch (PSI) for the entire sterilization time. The time will vary according to the contents and how the load is packaged.

- **Autoclave Tape** – indicator tape will turn black if exposed to temperatures of 250°F/121°C.

- **Biological Indicators (BIs)** – are the most accepted means of monitoring the sterilization process because they directly determine whether the most resistant microorganisms (e.g., Geobacillus or Bacillus species) are present rather than merely determine whether the physical and chemical conditions necessary for sterilization are met. Because spores used in BIs are more resistant and present in greater numbers than are the common microbial contaminants found on patient care equipment, an inactivated BI indicates that other potential pathogens in the load have also been killed. A biological indicator such as *Bacillus stearothermophilus* is used to verify the autoclave is properly sterilizing goods. The frequency of use of the BI in research, animal care and health care settings is determined by those facilities’ SOPs and accrediting agency requirements.
Observe expiration date on envelope and discard if expired. One type of indicator is the DUO-SPORE Envelope.

1. Remove the 2 biological indicator test strips from right pocket.
2. Label one strip with “TOP” and the other “BOTTOM”.
3. Place the “TOP” strip on top of the load and the “BOTTOM” strip either at the bottom or middle of load, whichever will require the greatest steam penetration.
4. Place envelope on top of the load since the Control strip is located in the left pocket.
5. After sterilization is complete, insert the 2 test strips into the right pocket of the envelope and seal. Fill out the Sterilization Report and take to Diagnostic Lab for evaluation. Note:

LOG BOOKS

- Entries must be placed in the log books each time the autoclaves are used. These records are used for maintenance/service schedules and the reporting of incidents, accidents and/or faults.
- The cycle records/printout for the autoclaves will be kept on the autoclave. When the printer paper is changed, the completed printout should be kept in the log book.
- The log should list the building, room number, and autoclave type/model.
- Entries should include
  - Operators name
  - Date/time/run type/ run and duration
  - If a repair was performed, the type of repair/parts replaced and service technician’s name should be entered into the log book.

IX. MAINTENANCE

- Discontinue use immediately if an autoclave is not working properly. Post a sign on the autoclave to notify others that the autoclave is not operational.
• Operators will examine door gaskets for damage each time the door is opened. If cracks or tears are noted, do not operate the unit and notify your supervisor immediately.

• Chamber

  1. Operators will examine the chamber for damage each time the unit is opened. If a large amount of water is noted, do not operate unit and notify supervisor immediately.

  2. If a strainer screen is present, examine strainer screen for debris and clean if necessary. Be sure to reinsert screen, if removed, before running a cycle.

• Operator is not to attempt to make repairs. Notify the Supervisor immediately.

X. EMERGENCY PREPAREDNESS

• If hurt, immediately call for Medical help!!!
• If clothing is soaked in hot water/steam, remove clothing and cool the injured part 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, procedures enacted to prevent future incidents, and the autoclave is deemed safe for operation.
• All incidents resulting in an injury to be reported to the Environmental Health & Safety Dept. Complete the required paper work the “First Report of Injury or Illness” form and seek medical attention.

Emergency Procedure

• 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 cleaning up spills. Contain the spilled material using appropriate clean up tools. Wait until the autoclave and materials have cooled to room temperature. Review the MSDS, if appropriate, to determine the protective equipment, spill cleanup, and disposal protocols that are necessary. Clean the equipment and work area in order to collect and remove all spilled materials. Dispose of the waste following the protocol appropriate for the material. If materials have been intermingled, follow the cleanup and disposal protocol for the most hazardous component of the mixture.
• Cracked glassware must be disposed of properly in the “Broken Glass” containers.
• Record the spill and cleanup procedure in the autoclave log.

XI. Training and Recordkeeping
Autoclave user training shall be given by the supervisor and the training documented. The sign-in sheet in this SOP may be used for this purpose.

REFERENCES

1. AMSCO Sterilizer Standard Operating Procedure. Department of Laboratory Animal Resources, UTHSCSA.
2. Autoclave Sterilization, Standard Operating Procedure. Division of Comparative Medicine, University of South Florida.