

STANDARD OPERATING PROCEDURES
DIVISION OF COMPARATIVE MEDICINE
UNIVERSITY OF SOUTH FLORIDA

SOP#: 1006.6

Date Issued: 10/98

Date Revised: 4/22

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TITLE:	Autoclave Sterilization
SCOPE:	Animal Care Personnel
RESPONSIBILITY:	Facility Manager and Technical Staff
PURPOSE:	To Outline the Proper Procedures for Autoclave Sterilization

I. PURPOSE

1. 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 pieces of equipment are used for sterilization in laboratory animal facilities, but the autoclave is the primary means of sterilizing supplies.
2. Despite built-in safeguards, an autoclave presents the possibility of serious injury to technicians from hot surfaces and from the release of live steam. It is important, therefore, that laboratory animal technicians understand the proper operation, limitations, and safeguards for sterilization by autoclaving.

II. RESPONSIBILITY

1. The Facility Manager ensures that all autoclave equipment is appropriately monitored, maintained in good working order, and available for research personnel as requested.
2. The Facility Manager ensures all research and technical staff are adequately trained and experienced in autoclave sterilization procedures.
3. Technical staff operating autoclave equipment ensures this procedure is followed.

III. PROCEDURE

1. Autoclave sterilization times and temperatures are dependent on the composition and nature of the items being autoclaved. **Minimum times** are usually 15 minutes at 250° F (121°C) or 5 minutes at 270° F (132.2° C).
2. Autoclaves should be set at **250° F when sterilizing microisolator caging**. Higher temperatures can damage polycarbonate caging.
3. Items to be sterilized are **loaded into the autoclave** in a manner so that nothing touches the inside of the chamber.

4. 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 the items.
5. **Small items** to be autoclaved **should be bagged or wrapped** in heat-resistant plastic, paper, or cloth.
6. **Sterilized supplies must be labeled** with date autoclaved/processed. Date of expiration is determined using the following criteria:
 - a. Sterile packs/items should be stored in clean, dry enclosed cabinets and drawers free from moisture and dust.
 - b. 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 for **1 year** providing the packaging remains dry and intact.
 - c. Sterile packs prepared by research or technical staff stored on open shelves have a reduced safe shelf life of **1 month** providing the packaging remains dry and intact.
 - d. The manufacturer generally stamps commercially prepared sterilized packs with an expiration date.
 - e. A shelf life of **3 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 date of receipt.**
 - f. 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.
 - g. Any package that is wet, torn, dropped on the floor, damaged in any way, or past its date of expiration will not be used. When such events occur, the contents should be removed, repackaged, and re-sterilized.
 - h. Sterilized static cages used for supplies have a shelf life of 1 month from the sterilization date.
7. Sterilization **indicator strips** (e.g., autoclave tape, steam indicator strips) should be used in each autoclave load. See **SOP # 1002** entitled **“Monitoring Autoclave Sterilization”**.
8. **Drinking water 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. The shelf life of sterilized drinking water is 1 month from the sterilization date.
9. When sterilizing materials using **autoclavable bags** (with the exception of biohazardous bags/materials), leaving the bag open during the autoclave process will facilitate steam penetrating the contents of the bag.
10. When autoclaving **biohazardous waste**:
 - a. Bags/containers should not be overfilled, half to three quarters full is recommended.

- b. Add water (e.g., approximately 1 cup) to the biohazard bag/container prior to sealing the container. This will aid in the generation of steam within the container during the autoclave process. Do not add water if there is a chance that biohazardous material may splash out of the container.
 - c. Seal container.
 - d. Biohazard bags/containers should be labeled with an autoclave indicator so that it can be readily determined that the container has been autoclaved.
11. For specific procedures of autoclave operation see the **SOP Index** for each autoclave make and model available for use in animal facilities.
12. When the autoclave cycle is complete and the pressure returns to zero, the door may be opened.
13. **Before opening the door**, staff members should don heat-resistant autoclave gloves. To prevent being scalded from the steam, move to the opposite side of the door opening. Open door slowly and, once opened, walk away from the direct line of the steam. After a few minutes, the autoclave materials can then be removed. **Items will still be very hot** for a while. Items should not be used until cool to the touch. Animal feed that has been autoclaved takes a lot longer to cool down than the container it was autoclaved in.
14. When removing water bottles, let the bottles of water sit in the autoclave for 10 minutes. The **bottles and the water will remain very hot** for a long while. It is recommended that the bottles not be used until the next day.
15. Staff members **encountering malfunctions** with the autoclaves should report it immediately to their supervisor and should not attempt to fix it themselves.

IV. SAFETY CONSIDERATIONS

1. Technicians can be seriously **injured from hot surfaces** and from the release of live steam. Use of the long-heat-resistant autoclave gloves is necessary.
2. **If steam escapes** through the gaskets while the autoclave is running, turn the autoclave off and get the supervisor or next in charge. **DO NOT OPEN THE DOOR.**

V. REFERENCES

1. American Association for Laboratory Animal Science. Training Manual Series, Volume II. Laboratory Animal Technician.

Approved:

Date: