FLORIDA ATLANTIC UNIVERSITY

Division of Research
Comparative Medicine

Original Date Released: 2/8/16 Version: 04 Date Last Revised: 2/26/18

SOP # 104	**Title: Autoclaving Procedures**

SCOPE: This SOP is applicable to all Comparative Medicine personnel

SOP OWNER: Certified Veterinary Technician

PURPOSE: To outline the procedures for autoclaving infectious items or items requiring sterilization prior to use.

LOCATION: All Vivaria

Approved by: Sylvia Gografe, D.V.M., Ph.D. Director Veterinary Services

References:

1. Product guide from temperature strip (or card) product and BI
2. Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, CDC, National Institutes of Health, Sept 2009, pg. 35 and 36
3. Occupational Health and Safety in the Care and Use of Research Animals, National Research Council, National Academy Press, 1997, Pressure Vessels, pgs 36, 44-50.
4. *SOP 110 Autoclave Effectiveness*
5. *SOP 111 Biological Indicator Processing*
6. *SOP 904 Autoclave Operation and Care*
7. *CM Form #059 Biological Indicator Log Sheet*
8. *CM Form #045 Autoclave Log*

1. Responsibilities

a. Laboratory Animal Technician/Autoclave Operator
   i. Adhere to procedures as outlined in this SOP.
   ii. Perform only procedures for tasks which have been trained.
b. Certified Veterinary Technician (CVT)
   i. Adhere to procedures as outlined in this SOP.

ii. Provide appropriate training including hands-on sessions to autoclave operator, document training and verify competency including knowledge of safety requirements.
iii. Oversee Quality Assurance Program including testing of autoclave effectiveness. Review Autoclave log sheets at least monthly for accuracy and collection of temperature strips

c. Facility Manager/Trainer Coordinator
i. Adhere to procedures as outlined in this SOP.
ii. Ensure procedures are followed as outlined in this SOP.
iii. Ensure appropriate training is provided to particular personnel depending on function/job description and assurance that Training record is signed.

d. Director/Veterinarian
i. Adhere to procedure as outlined in this SOP.
ii. Ensure appropriate training is provided to particular personnel and necessary resources are available.

2. Safety

a. Live steam, hot water or hot materials can cause serious burns. Best practice to avoid burns is to allow autoclaved items to return to room temperature.
i. When autoclaving water, strive to autoclave this item at the end of the day so that the contents have overnight to cool.
ii. To avoid steam exposure, keep a safe arms-length distance from the opening of the autoclave chamber.
iii. To avoid material handling burns, personal protective equipment (PPE) must be worn when unloading autoclaved items.
iv. Autoclave gloves must be used when handling hot items, and care must be taken to avoid burns when removing items from the autoclave. Inspect gloves prior to use for damage such as holes or rips in glove seams.
v. Autoclave sleeves are extensions of gloves and should also be used if personnel are required to reach into a hot chamber. The glove extensions provide protection to skin higher in the arm.
vi. Replace worn or damaged PPE.
vii. Heat resistant aprons and water resistant shoe (or boot) covers must be worn when handling hot water.

b. Upon starting the autoclave, hang a WARNING HOT MATERIALS sign on the autoclave door so that personnel in the area know the equipment is hot.
c. Upon removing items from a chamber on an autoclave cart or other storage area for hot materials, place a WARNNING HOT MATERIALS on top of the hot items.
d. In the event of an emergency, seek medical help immediately. In the event of any injury (steam or heat exposure or burn) contact your supervisor. Refer to *SOP 008 Reporting Accidents, Injury and Illness*.
e. If steam escapes the machine while the autoclave is running, turn the autoclave off at the panel, if accessible without danger of steam exposure, or the main power box if the panel is inaccessible due to steam emission and contact your supervisor immediately. DO NOT OPEN THE AUTOCLAVE DOOR.

f. **Note: Do not seal containers too tightly or they may explode.**

3. General Information

a. Autoclaves use high pressure and high temperature steam to kill microorganisms. Sterilization is a function of time, temperature and saturated steam. All three components are critical for a successful outcome.
b. To achieve sterilization, the autoclave load must be saturated with steam. Air pockets or insufficient steam supply will prevent effective sterilization.
c. Solid materials must be packed loosely. There must be ample room between the bags, trays and other containers so as to not impede steam circulation.
d. Autoclave or sterilization indicators are test tools used to verify the sterility of autoclaved materials or water. They come as Biological Indicator (BI) or chemical indicators. Chemical Indicators are available as temperature strips or temperature tape. See *SOP 110 Autoclave Effectiveness* for more information.
    i. Autoclave Indicators have different purposes. Therefore, it is important to pay attention to which type of indicator has to be used.
e. Ensure that materials being autoclaved are heat/pressure resistant including glass and plastics. Never autoclave:
    i. Oils, waxes, flammable materials such as alcohols, formalin, acetic acid
    ii. Radioactive materials
    iii. Sharps prior to disposal in a sharps disposal container
    iv. Dried bleach and bleach-associated materials due to fire or explosion risk
    v. Pathological waste like carcasses or tissues since those must be incinerated

4. Preparation for autoclaving infectious and selected toxin containing items

a. Supplies needed:
    i. Biohazard bags (Use only approved biohazard bags)
    ii. Permanent marker
    iii. Biological Indicators (BI)
    iv. Chemical Indicators; i.e. steam sterilization strips and temperature tape
b. Biohazard bags should not be overfilled; fill halfway to ¾ of the way.
c. Add a ½ cup of water to a container (cage, flask, bottle(s), etc.) inside the biohazard bag prior to sealing to aid in the generation of steam during the autoclave process. For safety purposes, do not pour water directly into the bottom of the biohazard bag. Do not add water if there is a chance that biohazardous material may splash out of the container.
d. Inside each biohazard bag, add:
    i. (1) steam sterilization strip
    ii. (1) biological indicator
        1. To be added once a month
        2. Will be in a pack for easy handling
        3. Label pack with:
            a. Date
            b. Autoclave location (i.e. building)
        4. Label bag and/or temperature tape with "BI" to indicate BI added to load

e. Do not seal containers such as large bottles of water tightly or they may explode.
f. Gooseneck (twist the top of the bag in a circular motion and fold over to create a bend or "gooseneck") the biohazard bag, but not tightly.
g. Wrap temperature tape around the neck of the red biohazard bag and write the date on the tape in permanent marker. The biohazard bagged items are now ready to be autoclaved.
h. Place the items to be autoclaved in the chamber as described in *SOP 110*.

5. Preparation for autoclaving items requiring sterilization prior to use

a. Supplies needed
   i. Surgical drape or autoclave pouch
   ii. Permanent marker
   iii. Scissors
   iv. Chemical Indicators; i.e. steam sterilization strips and temperature tape
b. **Safety reminder – when preparing items for autoclaving, do not seal containers too tightly or they may explode.**
c. Water bottles are filled about ¾, loosely capped, and placed inside an empty rat cage or in a bottle rack.
d. Cages are filled with bedding and set up with a nestlet, food hopper, lid, and cage card holder.
   i. To each load of cages, add/use:
      1. Temperature tape to lids
         a. On each individual cage or a stack of cages
         b. On two places at the front and back of the cage top to securely tape it shut.
         c. Avoid using excessive amounts of temperature tape on caging as this creates a sticky residue which is difficult to remove.
      2. (1) Steam sterilization strip
e. Surgical tools (e.g. scissors, needle drivers) need to be opened fully and placed inside an autoclave pouch or if situated in a surgical tool box draped with surgical drape.
   i. To each pack/box, add:
      1. Use temperature tape on the outside for closing, with a permanent marker write:
         a. Date of autoclaving
         b. Operator initials
      2. (1) Steam sterilization strip
f. Place the items to be autoclaved in the chamber as described in *SOP 110*.

6. **General Autoclave Procedures**

a. See *SOP 904 Autoclave Operation and Care* for operation instructions.
b. Process bottles on a liquid cycle at 121ºC for 30 minutes.
c. Process non-liquid items on a dry cycle at 121ºC for 30 minutes.

d. Process items containing toxins (non-heat stable) following recommendations by EH&S based on Pathogen Safety Data Sheet (e.g. Pertussis Toxin 121ºC for 60 minutes).
e. Check the print-out from the recorder to see if time and temperature were attained and if not re-autoclave the load, if necessary in another autoclave.
f. Collect and/or record sterilization indicators (i.e. temperature tape, steam sterilization strip, biological indicator) during unloading as described in *SOP 110.*
g. If the chemical indicator(s) (i.e. temperature tape, steam sterilization strip) did not change color**, the entire load will be removed, repackaged with new indicator(s), and re-sterilized**.
h. Alert a supervisor to investigate malfunction.
i. Any package that is excessively wet, torn, or damaged in any way should be **removed, repackaged with new indicator(s), and re-sterilized.**
j. See *SOP 111 Biological Indicator Processing*.

7. **Record Keeping**
   a. Record each autoclave run on the *Autoclave Log* notating*:*
      i. Date
      ii. Operator initials
      iii. Type of load run (i.e. Quarantine, BSL2, Lab Supplies, Surgery Packs, Sterile Caging/Water, Quality Assurance, etc).
      iv. PSI programmed
      v. Temp programmed
      vi. Time programmed
      vii. PI/Protocol
      viii. Temperature tape results
      ix. Steam sterilization strip results
      x. If BI added to load
      xi. Any comments, BI results (after processing BI according to *SOP 111*) or corrective actions taken
   b. The person opening the bag or container labeled "BI" is responsible for looking for and returning the pack from inside the bag to the CVT for processing.
   c. CVT to record all results from the BI live culture vial in both the *Biological Indicator Log Sheet & Autoclave Log*.
   d. Log any failed temperature tape, steam sterilization strips or failed BI tests from the autoclave on the building's *Health and Environment Check Sheet* and notify supervisor.
   e. Autoclave log sheets:
      i. Are to remain readily accessible for potential IACUC inspections for a period of one full year.
      ii. The first six months of the past calendar year can be filed after the first six months of the current calendar year are completed.
      iii. More than one year old may be archived.
      iv. Older than three years may be destroyed.

Review Date	Revision Date	Revision Number	Description of Revision
04/20/17	04/20/17	104.2	Including CVT responsibilities, adding to safety and recordkeeping section, new section "general information", removing detailed information regarding autoclave indicators (new SOP)
2/1/18	2/12/18	104.3	Added References, update Preparation for Autoclaving & update Record Keeping
2/26/18	2/26/18	104.4	Add procedures for destroying relevant toxins used in animals