



**Division of Research
Comparative Medicine**

Original Date Released: 5/28/17

Version: 02

Date Last Revised: April 12, 2018

SOP # 110**Title: Autoclave Effectiveness****SCOPE:** This SOP is applicable to all Comparative personnel**SOP OWNER:** Certified Veterinary Technician**PURPOSE:** To outline the procedures for assessing effectiveness of autoclave performance**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, pg 36, 44-50.
4. *SOP 104 Autoclaving Procedures*
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 Sheet*

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.
 - i. Heat and water resistant (rubber) aprons and water resistant shoe (or boot) covers must be worn when handling hot water.
- b. Upon opening a chamber, place a WARNING HOT MATERIALS sign on the autoclave door so that personnel just arriving 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 WARNING 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.
 - i. Autoclave Indicators have different purposes. Therefore, it is important to pay attention to which type of indicator has to be used.
- e. A Biological Indicator (BI) is an approximately ½ inch tall and 1/8 inch round glass ampule (or vial) filled with live bacterial spores. Once the vial is autoclaved, the goal for the operator is to incubate the bacteria after the ampule has been sterilized. If the incubated live culture displays growth of the bacteria, the autoclaved load did not achieve sterilization.
- f. Chemical Indicators are available as steam chemical integrators (i.e. 3M Comply SteriGage), temperature strips or temperature tape.
 - i. Steam chemical integrators - a tool used to measure time, temperature and the presence of saturated steam. The steam chemical integrators are chemical indicators consisting of a paper wick and a steam and temperature sensitive chemical pellet contained in a paper/film/foil laminate. The chemical pellet melts and migrates as a dark color along the paper wick. The migration is visible through a window marked REJECT or ACCEPT. The extent of migration depends on steam, time, and temperature.
 - ii. Temperature strips - a tool used to measure time and temperature of an autoclave load. The temperature strip will turn from a light color to a dark black once the targeted temperature has been achieved for a designated period of time. Temperature strips are printed on paper or card stock.
 - iii. Temperature tape - a tool used for easy visibility to see if the autoclave reached at least 180 degrees at one point. This adhesive masking tape changes from no color to a striped tape when exposed to a 180 degrees flash-point. However, it is important to understand that it does not confirm sterility since it doesn't confirm sustained high heat only a flash-point.
- g. 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. Procedures

a. Autoclave Indicator Storage and Preparation

- i. The bulk of autoclave indicators will be stored in the lab Bldg 71/Rm 220.
- ii. An appropriate number of chemical and biological indicators will be available in each facility in rooms where needed, e.g. the quarantine rooms, BSL2 housing areas and dirty cage wash rooms.
- iii. The CVT is responsible for ordering and distributing autoclave indicators.
- iv. Unopened and resealed packages need to be stored in a dry condition (humidity best <50%) at room temperature (59-86°F). Do not refrigerate or freeze. Do not expose to direct sunlight or UV light.
- v. All products have an expiration date that applies to unopened packages.
- vi. Never use any autoclave indicator that is expired. Always check expiration date before using indicator(s).
- vii. Use permanent marker to label BI packet with necessary information prior to autoclaving. This includes:
 1. Date
 2. Autoclave location (i.e. building)
 3. Label bag and/or temperature tape with “BI” to indicate BI added to load
- viii. When autoclaving water, place clear transparent tape over the temperature strip to protect the information recorded with the permanent marker. Steam could blur the recorded information if transparent tape is not used to protect the writing.
- ix. 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.

b. Autoclave Indicator Placement in Loads of Infectious Items

- i. At least one bag to every autoclave load (3-4 bags of caging) will have a BI. Bags containing a BI will be labeled with a permanent marker with the initials “BI”. The bag labeled “BI” should be placed in the most central part of every autoclave load.
- ii. A temperature strip will be added to the outside of the biohazard bag. This is a very visible indicator that time and temperature have been maintained long enough to open the inside of the autoclaved bag once it is cooled to retrieve the live culture ampule.
- iii. Add a second temperature strip in another OUTSIDE strategic location.
- iv. 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. Wrap temperature tape around the neck of the red biohazard bag.

c. Autoclave Indicator Placement in Loads of Items to Be Sterilized Prior Use

- i. A BI will be placed in the autoclave load at least once weekly for frequently used autoclaves, monthly for infrequently used autoclaves or in each load for less frequently than monthly used autoclaves. Position the BI at the slowest point of heating (e.g. center of load) either within a container or tape it to the outside of an item.

- ii. In each autoclave load place temperature strips in at least one container and one outside a container strategically located within the load. The container with the INSIDE temperature strip must be labeled outside with “Indicator”.
- iii. On complete cage set ups to be autoclaved, lids are taped shut using temperature tape on one place at the front of the cage top.

d. Assessment of Autoclave Indicators and Follow Up

- i. Allow the load to cool before unpacking.
- ii. Collect the BI from the load and process it as soon as possible with a control BI as per *SOP Processing Biological Indicator*.
- iii. Contents of this autoclaved load of infectious items should not be unpacked until the results of the BI(s) are negative. If the test results are positive then the contents of that autoclaved load must be processed again.
- iv. Contents of
- v. It might not always be possible to incubate the BI(s) and wait for test results before using items that require sterilization before use. If content of an autoclave load had been released for use and the BI test results are found to be positive (i.e. growth of bacteria occurs) later, any effort has to be made to trace the autoclaved items of this load for re-sterilization.
- vi. When unloading the autoclave check each temperature tape on sterilized items to confirm that it has changed from no color to a striped tape indicating that 180°C had been reached at least for a short time. If the tape has not changed don't unpack the autoclave instead **the entire load will be removed, repackaged with new chemical/biological indicator(s), and re-sterilized.**
- vii. Retrieve the temperature strip(s) from the outside of the central package to verify the strip has turned completely black. This means the temperature was maintained for the sufficient length of time to achieve sterilization.
- viii. If the temperature tape strip does not turn completely black then the autoclave did not achieve sterilization. Reprocess the autoclave load with a new temperature strip from the same or a different box if possible.
- ix. Temperature strips within packages will be collected by the technician when the item is opened for use. When viewing the temperature tape marked “INSIDE”, look for the tape to be black in color. If the tape is not black, do not use the contents, repackage and autoclave the item(s) again until sterilization can be verified by viewing a black temperature tape strip. Inform your supervisor.
- x. For repeat failures of biological indicators, alert the FM and CVT to investigate the malfunction.
- xi. Any package that is excessively wet, torn, or damaged in any way should be **removed, repackaged, and sterilized again.**

5. Record Keeping

- a. Record each autoclave run in the *Autoclave Log*, note whether the run was for infectious items or items requiring sterilization prior to use. Temperature strips

are each taped in the autoclave log. Each load will have two temperature strips attached in the log.

- b. Each OUTSIDE temperature tape is taped to the log sheet at the time the load is unpacked. INSIDE temperature tape items may take some time to be returned to the log book as frequency of use of items may vary. The person opening the bag or container labeled “Indicator” is responsible for looking for and returning the temperature strip from the inside of the package to the log book.
- c. Record all results from the BI live culture vial in the *Autoclave Log*.
- d. Log any failed temperature strip or failed BI tests from the autoclave on the *Health and Environment Check Sheet* and notify supervisor.
- e. The first six months of the past calendar year can be filed after the first six months of the current calendar year are completed.
- f. Autoclave log sheets for a period of one full year are to remain readily accessible for IACUC potential inspection; logs more than one year old may be archived. Records older than three years may be destroyed.

Review Date	Revision Date	Revision Number	Description of Revision
4/12/18	4/12/18	110.2	Change to PPE