FLORIDA ATLANTIC UNIVERSITY

**Division of Research**
**Comparative Medicine**

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| **SOP # 111** | **Title: Processing Biological Indicators** |
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**SCOPE:** This SOP is applicable to all Comparative Medicine personnel

**SOP OWNER:** Certified Veterinary Technician

**PURPOSE:** To outline the procedures for autoclaving for processing biological indicator *Bacillus stearothermophilus* or *B. subtilis* used in determining autoclave effectiveness.

**LOCATION:** All Vivaria

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

**References:**

1. The Biosafety in Microbiological and Biomedical Laboratories, 5th Edition
2. SOP110: Autoclave Effectiveness
3. SOP119: Incubator Use and Maintenance for Biological Indicators
4. BI sample product sheet(s) and/or Safety Data Sheet for each type of BI
5. BI incubator product sheet(s)
6. Autoclave Log Sheet (CM#045)
7. Biological Indicator Log Sheet (CM#059)
8. Health and Environment Check Sheet (CM#008)

**1. Responsibilities**

a. Laboratory Animal Technician (LAT)/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. Collect biological indicator (BI) sample, complete incubation process and recordkeeping using the procedures described in this SOP.
iv. 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 (FM)
   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.
   iv. Correct equipment problems or staff training leading to non-compliance.
d. Training Coordinator
   i. Schedule training at frequency to maintain competency to SOP.
   ii. Ensure appropriate training is provided to personnel.
e. Director/Attending Veterinarian (AV)
   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. Caution should be used when pressing the inner glass tube of the biological indicator tube. The inner tube is a fragile glass and is intended to be broken with light pressure; however, excessive pressure can cause the outer tube to break as well. When the outer tube is broken, bacteria and glass are potential sources of contamination or injury.
b. Personnel should always wear PPE relevant to this task: lab coat, gloves and eye protection.

**3. General Information**

a. Steam sterilization (autoclaving) is one of the most effective methods for decontaminating biohazardous or quarantined materials. Live culture BI are considered the "gold standard" or most effective method for verification of autoclave performance.
b. BIs are spores of *Bacillus stearothermophilus* (and *B. subtilis* in some products) and must be incubated after autoclave processing @ 56-60$^{o}$C according to incubator operating instructions.
c. Review *SOP119: Incubator Use and Maintenance for Biological Indicators* before operating steam incubator.
d. The following items need to be available at all times:
   i. Autoclave log sheet
   ii. Autoclave log book
   iii. BI equipment – incubator to meet BI product specifications
   iv. BI test product – purchased to meet processing time and bacterial count levels
   v. PPE: Autoclave gloves and eye protection.

**4. Procedures**

a. Operator verify autoclave occurred for a minimum of 30 minutes unless validation data demonstrates that less time is sufficient (or more time is required) for decontamination.
b. Add task to monthly equipment duties for each set of autoclave parameters used.
c. Follow *SOP110: Autoclave Effectiveness* to produce a BI for incubation.
d. Collect a cooled and labeled BI from autoclave.
e. Check labeling date and time of autoclave start cycle to make sure steam did not impact the labeling on the ampule. If so, re-label with date and time of autoclave start before delivering the sample for processing. This information will be placed in BI records. See recordkeeping (Section 6) below.
f. Deliver sample to the appropriate location (lab or procedure room depending on facility) for processing.
g. Once in the lab were the incubator is located, gently press on the tube to break the inner glass ampule of the BI which has been in an autoclave cycle and place in incubator.
h. Gently press on the tube of a second ampule which has not been inside an autoclave, to be processed as a control, label with date and "control" and place in incubator as well.
i. Turn incubator switch to “on”. Follow the manufacturer’s instructions for operation of the incubator.
j. Allow the BI to process the required amount of time (1 hr, 3 hrs, 24-48 hrs) for the selected BI product.
k. Collect the sample from the incubator at the end of the processing time.
l. Review colors of both the control and the autoclaved ampule(s). See the product manual for the appropriate colors for the control sample and the autoclaved sample.
m. Record BI results on the *BI Log sheet*.
n. Record outcome on the *Autoclave Log sheet* at the appropriate vivarium.
o. Notify CVT and FM of failed results of BI incubation failure and re-process materials; especially before handling contents inside biohazard bags.
p. If outer tube of any BI (autoclaved or non-autoclaved) breaks contain spillage, mark area and inform all personnel working in the area of potential hazard. Then follow EH&S established containment and cleanup procedures.

5. **Record Keeping**

a. Document all validation techniques for decontaminating biohazardous materials and sterilizing tools, caging and supplies on the Autoclave Log sheet.
b. Log any failed temperature strip or failed BI tests from the autoclave on the *Health and Environment Check Sheet* and notify CVT and FM.
c. Records should be reviewed regularly by the CVT, the FM and possibly at least semi-annually by the IACUC or EH&S personnel.
d. The first six months of the past calendar year can be filed after the first six months of the current calendar year are completed.
e. 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 |
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