| STANDARD OPERATING PROCEDURE | | | | |
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| Ragon Institute of MGH, MIT and Harvard | Autoclave Validation Procedure | | **SOP#** | TC-SOP-007 |
| | Originated by: | Alicja Trocha | Date: | 23 November 17 |
| | Laboratory: | Walker Laboratory | Pages: | 1 of 7 |
| | Approved by: | Alicja Trocha | | |

## I. PURPOSE

The purpose of this procedure is to outline the uniform procedure for validation of all autoclaves actively being used at Ragon Institute laboratories.

## II. SCOPE

This procedure applies to all employees, students, contractors and visitors that enter and/or work in the Ragon BSL2+ Institute laboratories.

## III. SAFETY

This protocol needs to be carried out in the BSL2+ laboratory following all BL2+ regulations.

## IV. RESPONSIBILITIES

A. The Ragon Institute **Managers, TC Room Leaders and all Laboratory Principal Investigators** are responsible for the overall implementation of this procedure and ensuring compliance.
B. **The Ragon Institute Managers are** responsible for periodically reviewing the application and maintenance of this procedure, and initiating any updates to this procedure.
C. **All employees, students, contractors and visitors** are required to follow this procedure. Non-compliance with this procedure will result in the assignment of a corrective action plan.

## V. PROCEDURE

Autoclaves used for biohazardous waste sterilization in TC rooms shall be challenged periodically to verify effectiveness of the equipment and process. 3M™ Attest™ Biological Indicators shall be used as challenge agents.

(Note: All biohazardous waste at the Ragon Institute is ultimately shipped offsite by a certified waste vendor for further sterilization and disposal.)

Biological Indicator (BI) Product Description: 3M™ Attest™ Biological Indicators are designed for monitoring steam sterilization processes. 3M™ Attest™ Biological Indicators are vials containing ampuled growth medium and filter paper with spores of Geobacillus stearothermophillus. The ampuled growth medium has a pH indicator that changes color during incubation from purple to yellow in the event of test organism growth. The BI label also has a chemical indicator that changes from rose to brown when exposed to the steam sterilization process.

BI Storage: 3M™ Attest™ Biological Indicators can be stored under normal conditions: 15-30°C (59-86°F), 35-60% relative humidity. Do not store near chemicals. BIs have a shelf life of 24 months.

**Autoclave Validation Frequency:** Testing shall be conducted every 3 months for the TC Room autoclaves.

**Autoclave Validation Records:** The template provided as Appendix A shall be used to document autoclave validation test results. Records shall be maintained in binders and stored in each TC Anteroom.

**Autoclave Validation Instructions:**

**Supplies:**

- Autoclave Validation Records (Appendix A)- each floor at Ragon Institute have a binder.
- 2 BIs per autoclave (1 dry cycle, 1 liquid cycle)
- 1 BI for positive control (from same manufacturing date and lot # )
- 1 dry cycle mock waste load per autoclave (autoclave bag with uncontaminated dry waste)
- 1 liquid cycle mock waste load per autoclave (metal bucket with uncontaminated water)

**Procedure:**

1. Identify each BI by writing the autoclave location, date, and "L" for liquid cycle or "D" for dry cycle on each BI label. Identify the positive control BI by writing "PC" and the date.
2. Complete the following steps 4-8 for each autoclave and cycle.
3. Place BI in center of mock waste bag or bucket of liquid.
4. Autoclave the mock waste according the TC Room Autoclave Instructions (Appendix B).
5. After sterilization, allow the mock waste to cool.
6. **Put safety glasses and disposable gloves** and remove BI from mock waste load. Wait an additional 15 minutes to ensure BI is cool **(risk of BI bursting during the activation process if not cool).**
7. Check the chemical indicator on the BI label for color change from rose to brown. **Discontinue test and consult EH&S if color change does not occur.**
8. Activate each BI using the following procedure (including positive control):
    a. Using the corresponding BI incubator, position bottom of BI into the incubators heating block so the BI is at an angle of approximately 45°.
    b. Push the BI straight back to crush the growth media ampule. Ensure the cap remains above the metal block (this step activates the indicator).
    c. Push the activated indicator down to seat it in the metal block. Ensure the cap remains above the metal block.
9. Incubate all BIs for 48 hours at 56 $\pm$ 2°C for 48 hours.
10. After incubation, ensure a color change from purple to yellow is visible in the positive control BI (this demonstrates viability of the batch of BIs).
    a. If color remains purple, **the batch of BIs is not valid and retesting with a new batch is required.** Complete each Autoclave Validation Record by indicating (-) under the QC Results/control, "n/a" under Test Passed, and include printed name and signature.

   b. If color changes to yellow, indicate (+) under QC Results/Control on each Autoclave Validation Record.

11. Check the processed BIs for no color change (processed BIs should remain purple).
   a. If color changes to yellow, the sterilization was inadequate. The corresponding autoclave and/or process will need to be assessed. Complete the corresponding Autoclave Validation Record by indicating (+) under the QC Results/Challenge, "**X**" under Test Passed, and include printed name and signature.
   b. If color remains purple, the corresponding autoclave has been validated. Complete the corresponding Autoclave Validation Record by indicating (-) under QC Results/Challenge, "✓" under Test Passed and include printed name and signature.
12. Dispose of BIs in biohazard waste boxes.

**TC Room Autoclave Validation Record**

**AUTOCLAVE IDENTIFICATION**

| | | |
|---|---|---|
| **Brand:** | **Model #:** | **Serial #:** |
| **Location:** | | |

**TEST RECORD**

| **Date** | ***Process Parameters*** | | | | **QC Results (+/-)** | | **Test** | **Printed Name** |
|---|---|---|---|---|---|---|---|---|
| | **Cycle (Liq./Dry)** | **Time (Min.)** | **Pressure (PSI)** | **Temp. (˚C)** | **Control** | **Challenge** | **Passed** | **Signature** |
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*TC Room Autoclave Instructions*

(Before first time use please contact TC room leader for proper training)

1. Press OPEN DOOR: Once to seal, twice to unseal and open.
2. Press SELECT CYCLE button on view screen.

   Cycle P1 – Wrap Goods (material wrapped in a bag).
   Cycle P7 – Hard Goods (pipets, pipet tips, etc.).
   Cycle P13 – Liquids (any cycle with liquids).

When LIQUIDS are present in the autoclave, you may only use the LIQUIDS CYCLE (P13)

3. Press ENTER to lock in cycle.
4. Press START: the door will seal if not sealed manually.
5. The PROCESS COMPLETED will illuminate green when cycle is complete. It will illuminate red if the cycle was aborted.
6. If the process failed, the PROCESS FAILURE LED will illuminate.
7. Press OPEN DOOR button and let cool for ~30 minutes before removing.
8. Remove your articles.
9. Dispose of liquids in sink between bay 14 and bay 15.
10. Dump remaining solids into red biohazard waste box.
11. Tape and label biohazard box with the following:
    a. “Please Remove and Replace”
    b. “Room #”