RUTGERS UNIVERSITY Office for Research	CMR STANDARD OPERATING PROCEDURE	
TITLE: Validation & Sterility testing for Flexible-Film Isolators		
SOP Category: Gnotobiotic	**CMR SOP #: 4.13**	**Page: 1 of 4**
Effective Date: 10/29/24	**Approval: *LaTisha V. Moody, DVM, DACLAM***	
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SCOPE:

This document describes the procedures to be followed when performing routine testing in the Gnotobiotics Core. This SOP applies to all trained Gnotobiotics Staff (at the Rutgers University facilities.

OBJECTIVE:

The objective is to ensure flexible-film isolators are sterile before entry of animals or supplies.

TERMINOLOGY

- **Manometer** – a device that measures the pressure of a gas or liquid in a confined space, relative to atmospheric pressure. This device allows a user to detect a leak in the flexible-film isolator system. If the pressure is less than 1 inch of water, then that is indicative of a leak.

PROCEDURES:

Leak Testing the Isolator

1. The isolator must be tested for leaks before it is sterilized using a Digital Manometer, that is used to measure the air pressure inside the isolator.
2. Put the port cap on and seal one of the two nipples with a Neoprene stopper attached to a manometer.

3. The isolator is inflated through the other nipple in the cap using the compressor and the atomizer without Clidox solution until the gloves are completely inverted and are standing out from the isolator horizontally.

4. One of the nipples is plugged using a rubber stopper. The second nipple, keep the Neoprene stopper attached to the manometer.
5. Turn on the digital manometer. Leave the isolator undisturbed for at least 12 hours. The air pressure must be above 1.00 inches of water (InH02).

Validate Sterilization Process of the Flexible Film Isolator

1. The isolator should be appropriately assembled according to the manufacturer's directions.
2. Isolators **must** be scrupulously hand cleaned before being sterilized, including new isolators recently purchased from major vendors.
3. Isolators should be thoroughly inspected for damage, especially filters, gloves, sleeves, and vinyl flexible film parts.
4. The isolator must be tested for leaks before it is sterilized.
5. Collect the following items to be placed in the isolator:
    - Floor mat
    - Plexiglas cage shelves

- Dressing forceps
- Two 12” inner port cap
- 2 rubber bands

6. Prepare 1 L of 1:3:1 Clidox solution and fill spray atomizer bottle with the solution.
7. Place spray atomizer into the main chamber isolator then use the nipple cap to introduce the atomizer tube through the nipple opening.
8. Ensure the stopper tube is attached to the nipple opening and seal it, the second nipple opening seal with a rubber stopper.



9. Using the atomizer, spray the isolator gloves, inside the inlet and outlet air filter openings, and around the glove rings and the junction of the chamber and port. Hold spray atomizer close to joints to blow sterilant thoroughly into all joints. All surfaces should be saturated.

10. Thoroughly wet all surfaces of the floor, mat, cage shelves, and inner port cap dunk rubber bands, forceps, and stoppers in the isolator.
11. Remove the atomizer from the main chamber of the isolator and place it in the entry port.
12. Place the inner port cap on the port and apply the rubber band. Take off the outer port cap to remove the atomizer and tubing out of the entry port. Put the outer port cap back and seal with two rubber stoppers.
13. Let the isolator stand at least overnight.
14. After 24 hours, pop the mylar of the inner and outer filters with the long forceps and plug in the motor so the air flow turns on.
15. The following day, start with the first connection containing cages, paper towel, swabs and 2 media tubes Fluid Thioglycollate medium (FTG) and Sabouraud Dextrose (SD).
16. Use swabs only to collect samples in the first connection from the surface inside the isolator. For subsequent connection sterile water will be used to moisten the swabs.
    - Swab #1: the top of the isolator
    - Swab #2: the sides of the isolator
    - Swab #3: the floor of isolator and on any objects on floor
    - Swab #4: around the port and corners
17. Ensure the media tubes are tightly sealed and place swabs inside of them.
18. Place the media tubes into the entry port.
19. Place the inner port cap to seal off the isolator.
20. Remove the outer port cap and remove media tubes and any trash. Re-sterilize the inner port using Clidox before replacing the outer port cap with black rubber band and clamp.
21. Swabs are incubated for 14 Days (FTG at 37° C and SB at room temperature).