RUTGERS UNIVERSITY Office for Research	CMR STANDARD OPERATING PROCEDURE	
TITLE: Gnotobiotic Facility Operations for Flexible-film Isolators		
SOP Category: Gnotobiotic	**CMR SOP #: 4.05**	**Page: 1 of 8**
Effective Date: 9/17/24	**Approval: *LaTisha V. Moody, DVM, DACLAM***	
Revisions: 8/22/23, 9/05/2024		

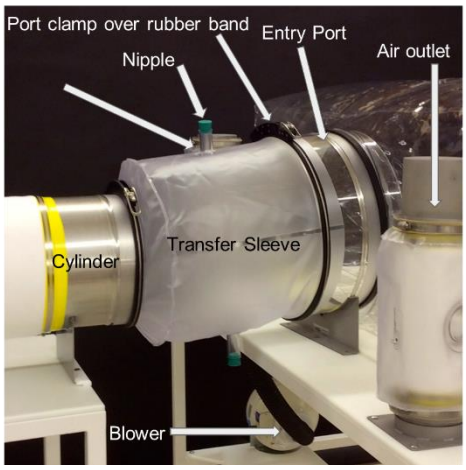
SCOPE:

This document describes the procedures to be followed within the gnotobiotic facility to maintain germ-free status of the colony. This SOP applies to all Gnotobiotic trained Staff only at the Rutgers University Gnotobiotic facilities.

OBJECTIVE:

The objective is to operate equipment safely and securely using the CBC flexible-film isolators. Whenever the port entrance is accessed, the port will be re-sterilized when all activities have been completed and a sample is removed from the isolator. *This will describe the process to follow in the event of an emergency situation such as when removing animals when a glove is damaged*.

TERMINOLOGY:



PROCEDURES:

Don Personal Protection Equipment (PPE)

1. Sterile PPE is not typically required for work outside of the flexible-film isolators.
2. Don chemical respirators only when hand spraying or wiping surfaces with 1:3:1 Clidox sterilant.

Bringing Glass Water Bottles into the Isolator Through the Port

1. Gather supplies needed:

800 ml spray bottle containing 1:3:1 Clidox
Atomizer with bottle of Clidox
Yellow vinyl tape
1 timer
5 autoclaved clear 1 L water bottles

2. Whenever opening an inner or outer port door or cap, operators should *always* verify that the opposite cap or door is secured. If both are accidentally opened at the same time, the isolator will be contaminated.
3. Don your chemical respirator.
4. Open the outer port by unclamping the port clamp and rolling the black rubber band back.
5. All interior surfaces of the port and the surface of the 1L water bottles are thoroughly wetted with sterilant using the spray bottle. Ensure removal of any visible debris and clean surfaces very well. Ensure removal of any tape and tape residue from the bottles before sterilization commences.
6. Place bottles into the port and liberally spray with Clidox.

7. Remove tape around one nipple. Spray down the outer port cap including the outer edges and attach the outer port. Place the outer port cap onto the port with the seam side at the top.
8. Leave about an inch of the vinyl port cap before rolling the black rubber band over the edge. You want the black rubber band 2-4 mm away from the edge. Secure the outer port with the port clamp over the black rubber band.

9. Plug in the atomizer, turn pressure up to 35 psi and place atomizer nozzle into the open nipple. Push the port cap into the port to decrease the pressure and pull the trigger of the atomizer until port fills up with sterilant fog. Follow *SOP #4.06 Using the Atomizer for Sterilization*.
10. Set timer for 20 minutes for contact time.
11. Remove your chemical respirator.
12. After 20 minutes, put your gloved hands into the isolator gloves then remove the inner port cap rubber band and open the inner port cap.
13. Put a sterile paper towel under the port edge to catch any sterilant that may drip onto the isolator floor.
14. Bring water bottles inside and perform your normal duties.

Attaching the Transfer Sleeve to the Supply Cylinder and isolator port described in SOP #4.19 *Prepping, Autoclaving Cylinders & Attaching Transfer sleeve.*

Fogging (Sterilizing) the Transfer Sleeve

1. Refer to *SOP # 4.06 Using the Atomizer for Sterilization* to prepare the atomizer for use.
2. Plug in the air compressor and set the pressure to 35 psi.
3. Spray the nipple with Clidox first, then place the atomizer nozzle into the nipple opening and pull the trigger to fog the transfer sleeve.

a. *Note*: all isolator ports are 12” in diameter

4. Continuously spray into the transfer sleeve for about one minute. Make sure that there is a thick fog present throughout the sleeve, and it is firm to the touch.
5. Once the transfer sleeve is inflated, plug the nipple with the rubber stopper. Set the timer to 20 minutes and wait for the 20-minute contact time.

Bringing Cylinder supplies Into the Isolator

1. Put cotton gloved hands through the isolator gloves.
2. Put the yellow rubber gloves on which can be found inside the isolator.

3. Place 1-2 sterile paper towels underneath the inner port cap to catch any sterilant that spills into the isolator.
4. Remove the inner port cap and rubber band.
a. The isolator will quickly deflate once you pop the mylar or silicone cap on the cylinder.
5. Obtain the long metal forceps. Use the forceps to puncture the vinyl film on all sides and tear the film to create a large circular hole. For cylinders with collars, this step can be omitted.
6. Remove all the items from the cylinder. Place food into an empty clean mouse cage and organize your supplies. Ensure there are no sharp objects left on the isolator floor.
7. Take a swab and dip into a water bottle to moisten. Swab all the areas inside of the isolator such as around your gloves, air intake/outtake, around the port, isolator walls, isolator floor, and swab any supply surface if animals are not present.
8. Take 4-10 pooled fecal pellets in a PCR tube if animals are present in the isolator.
9. Clean the isolator floor with sterile paper towels and water once you have finished your tasks.

Bringing Live Animals into the Isolator

1. Gather supplies needed:

800 ml spray bottle containing 1:3:1 Clidox
Atomizer with bottle of Clidox
Yellow vinyl tape & scissors
1 timer
SPP cage or 1L glass bottle containing mice

2. Whenever opening an inner or outer port door or cap, operators should *always* verify that the opposite cap or door is secured. If both are accidentally opened at the same time, the isolator will be contaminated.
3. Don your chemical respirator.
4. Open the outer port by unclamping the port clamp and rolling the black rubber band back.
5. All interior surfaces of the port and the surface of the cage or glass bottle are thoroughly wetted with sterilant using the spray bottle. Ensure removal of any visible debris and clean surface very well with lots of sterilant and paper towels. Remember to remove the cage card holder from an SPP cage and place it into the isolator binder. If using a 1L glass bottle, ensure to spray the edges of the bottle cap very well.
6. Spray the cage bottom and sides, including the front of the cage before placing it into the port and liberally spray all sides of the cage with Clidox.
7. Liberally spray the outer port including the nipple before replacing the outer port cap. Clamp the outer port clap and roll the black rubber band back into place.

Remove the inner port rubber bands and cap before bringing the GF animals into the isolator through the port.

Cage Change Frequency & husbandry tasks

1. Allentown shoebox cages will be changed every 2-3 weeks for all GF mice inside isolators. Other cages may be changed more or less frequently depending on the condition of the cage. Any Flooded cage *must* be immediately changed.
2. All dirty bedding will be dumped into a paper bag and the cage bottom will be wiped clean with a scrapper and dry sterile paper towels.
3. Animals will be placed into a clean cage with no bedding.
4. Place new BioFresh bedding into the wiped clean cage about 0.5 cm deep of bedding. Re-use the EnviroPack unless it is visibly too dirty to re-use for all mice. Wipe down the mouse hut and place it into the clean cage for breeder cages or single housed animals.
5. Animals will be placed into a clean cage with new bedding.
6. Wire bars should also be wiped clean with a moistened paper towel if it is too dirty.
7. Fill the low-profile water bottles (16 oz) with sterile water or top off as needed.

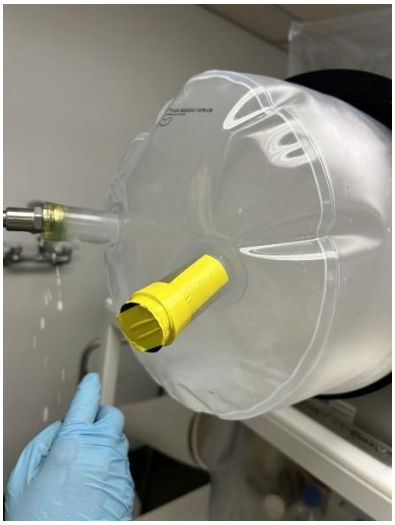
Removing Items out of the Isolator and Re-sterilizing Port after Exiting Items Out

1. Items that may be removed from the isolator include:
a. General trash (paper towels, old bedding)
b. Carcasses in paper bag or autoclavable pouches
c. Dirty cages, water bottles, fecal or swab samples

2. Whenever opening an inner or outer port door or cap, operators should *always* verify that the opposite cap or door is secured. If both are accidentally opened at the same time, the isolator will be contaminated.
a. The only exception would be if a cylinder is already attached to the opened port.
3. Wipe down wet surfaces inside the isolator and clean the isolator floor.

4. Open the inner port cap by removing the rubber band and taking the cap off.
5. Place all items that must be removed into the port entrance.
6. Close the inner port by placing the port cap and rubber band to seal the inner port.

7. Remove the outer port cap by unlatching the port clamp over the black port rubber band and rolling rubber band away. Remove the outer port cap, remove the items from the port, and dispose of items as appropriate.
8. Wipe off and clean any debris left inside the port using sterile paper towels.
9. Re-sterilize the port according to SOP # *4.06 Using the Atomizer for Sterilization*. **Clean the transfer sleeve and atomizer immediately after each use**. Ensure the isolator is fully inflated and record the date it was accessed, and record items entered or exited in the isolator binder.



Removing Live Animals out of the Isolator using Paper Bags

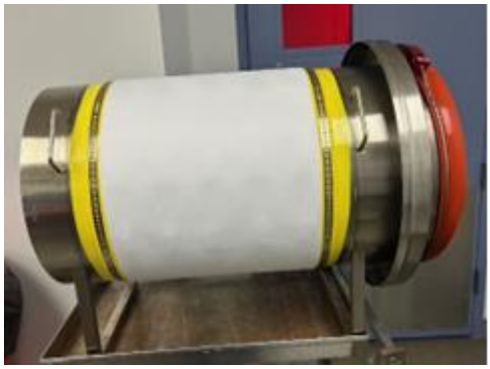
1. Whenever the isolator is accessed, a sample must be obtained from the isolator for surveillance testing. Collect a fresh fecal sample from a cage and put it in the fecal tube.

2. If a live animal needs to be removed quickly from the isolator, place it in a doubled paper bag or empty glass bottle. Moribund animals may be euthanized by cervical dislocation inside the isolator prior to removal by certified individuals.
3. Fold the top edges down so the mouse cannot escape.
4. Open the inner port cap by removing the rubber band and taking the cap off.
5. Place all items including the paper bag containing the mouse, into the port entrance.
6. Close the inner port by placing the port cap and rubber band to seal the inner port.
7. Remove the outer port cap by unlatching the port clamp over the black port rubber band and rolling rubber band away. Remove the outer port cap, remove the items from the port, and dispose of items as appropriate.
8. Wipe off and clean debris left inside port using paper towels.
9. Re-sterilize the port according to *SOP # 4.06 Using the Atomizer for Sterilization*.

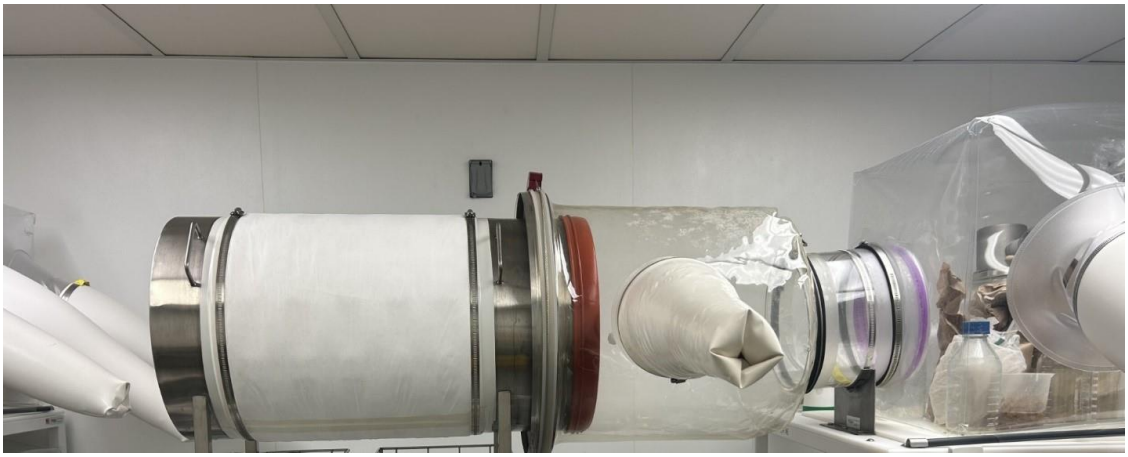
EMERGENCY SITUATION: Moving Animals out of the Isolator when Glove Breaks

1. Upon discovering a hole in the gloves or isolator, immediate action is required. The damaged glove should be securely tightened and submerged in a jar containing Clidox solution.


2. Proceed to autoclave the empty cylinder as per standard protocol for normal connections.


3. After autoclaving the cylinder, proceed with the connection process as usual as described in SOP # 4.19 *Prepping, Autoclaving Cylinders & Attaching Transfer sleeve*.



4. Once the sterilization cycle is complete, open the cylinder and begin loading it with cages containing animals. Please note that the cylinder has a capacity to store only 8 cages.
5. Gently close the cylinder using the red silicone cap, and detach the transfer sleeve from the isolator port, keeping it on the cylinder side.
6. Position the cylinder with the attached sleeve beside the new isolator, then connect the sleeve to the isolator port.
7. Spray clidox as you would for a standard connection and allow for a 20-minute contact time.
8. Remove both the isolator port cap and the red silicone cap. Open the isolator port cap and the red silicone cap.
9. Transfer the cages from the cylinder into the new sterile isolator, ensuring the animals are alive and completely safe throughout the process.
10. Collect fecal samples within one to two weeks to confirm the germ-free status.

Checking and Maintenance of the Mold Trap

1. Mold traps should be visually checked daily (Monday-Friday) and recorded on the activity log sheet.
2. Any growth in the mold trap should be marked with “+” symbol and initial. The gnotobiotic manager and facility supervisor should be notified immediately to evaluate the mold trap for contamination. If growth is noted over the weekend,

the on-call gnotobiotic member should be notified and they will contact the supervisors & managers.

3. Whenever food is entered into the isolator, a small handful of food pellets should be placed into the mold trap and topped off with water. Mold traps may become dry overtime, so water can be added to it as often as necessary to ensure mold trap is always damp.