RUTGERS UNIVERSITY Office for Research

CMR STANDARD OPERATING PROCEDURE

TITLE: Prepping, Autoclaving Cylinders & Attaching Transfer sleeve

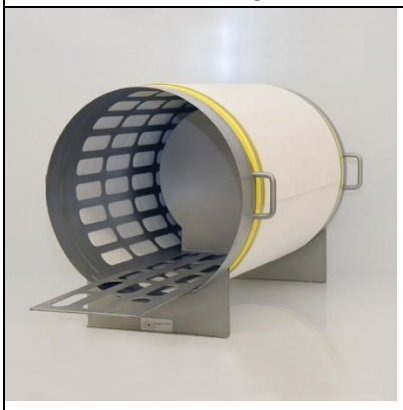
SOP Category: Gnotobiotic	**CMR SOP #: 4.19**	**Page: 1 of 9**
Effective Date: **9/03/2024**	**Approval: LaTisha V. Moody, DVM, DACLAM**	
Revisions: 8/08/24		

SCOPE:
This document describes the procedures to be followed when prepping and autoclaving cylinders. This SOP applies to all trained staff only at the Rutgers University Gnotobiotic facilities.

OBJECTIVE:

The objective is to ensure dry supplies are prepared inside of the cylinder for sterilization and describe the method of entering sterile supplies into the flexible-film isolator via cylinders.

MATERIALS:

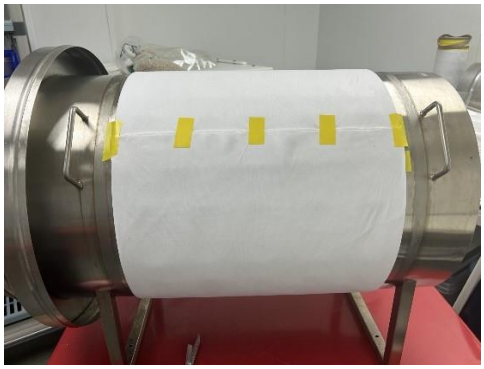
Supply Economy sterilizing cylinder w/ supply tray 12” or 18”	Feed sterilizing cylinder with collar 18” diameter	DW-4 filter media
		

PROCEDURES:

A. Wrapping the Cylinders

1. The cylinder wrapped with filter paper is only suitable for 30 uses. After reaching this limit, the cylinder must be re-wrapped.
2. Unwrap the cylinder and remove all residual filter paper and tape.

3. Send the unwrapped cylinder to the dirty cage wash area to be washed & sanitized.
4. We have 3 available sizes of filter paper DW-4 in the facility 12”, 15” and 18” wide.
5. Cut the filter paper to approximately 170 inches in length for both cylinders (economy and with collar)
6. Begin wrapping the cylinder with the cut filter paper, ensuring three even layers are applied and avoiding wrinkles.



7. Secure the end of the filter paper with yellow vinyl tape after the third layer.

8. Use yellow vinyl tape to secure both sides of the filter paper.

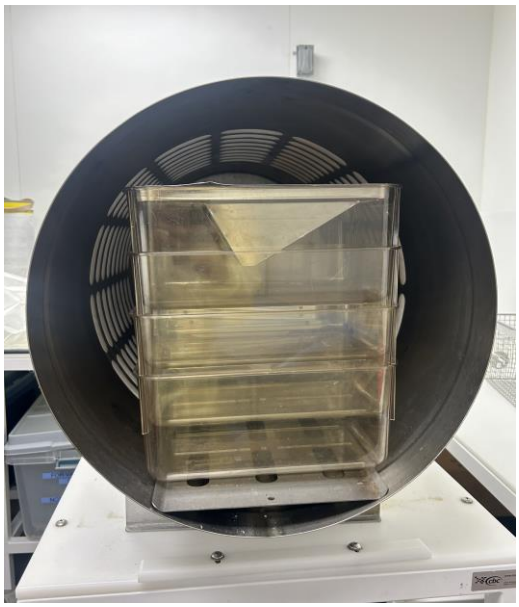
9. Secure the taped sides with a metal clamp, ensuring they are tightly secured using a Socket wrench screwdriver.

B. Preparing 18" cylinders for Dry Cycle Autoclaving

1. Supply Cylinder (gather items listed below):
    1. **Type A Set-up (New Isolators)**
        1. 18 wire bars
        2. 18 shoe box cages
        3. 8 reusable mesh bags (Earthwise) of bedding with 2 scroops using 1L plastic beaker of Biofresh bedding each. Tie a loose slipknot so bedding does not spill out (see image below).
        4. 1 reusable mesh bag of EnviroPAK's with 18 EnviroPACK's in bag
        5. 1 reusable mesh bag with Half pack of Wypall absorbable napkins

6. 2 reusable mesh bags (Earthwise) with 9 mouse water bottles in each pouch. If the bottle rims are sharp or damaged, do not use them. Empty bottles and tubes should remain uncapped.
7. 1 reusable mesh bag with 18 bottle twist tops. If bottle tops are damaged or sharp on the rim, do not use them.
8. 2 red or yellow mouse igloos in a reusable mesh bag.
9. 2 pairs of yellow rubber gloves.
10. 2 sharpened pencils in an autoclavable pouch
11. 10 empty paper bags
12. 20 fecal collection tubes in an autoclavable pouch
13. 10 small paper bags for carcasses or animal transfer from isolators into IVC cages.
14. 18 metal cage tags
15. 1 scraper tool for cleaning cages
16. One 500ml beaker for the mold trap

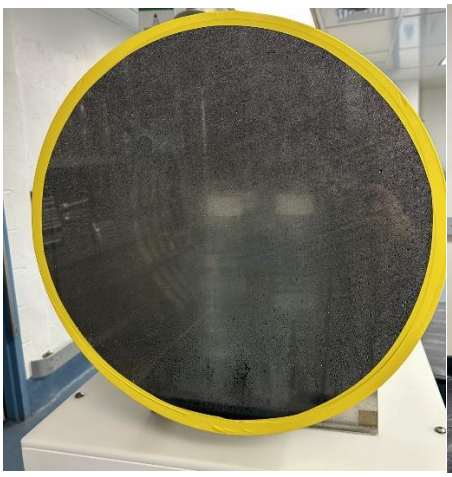
2. Place all the items inside of the 18” cylinder in the following **Type A Set-up** configuration:



3. This is the **configuration of the Feed cylinder**.
    1. The gnotobiotic bag of food will be evenly distributed onto all 4 feed trays into a single layer using clean gloves.
    2. Place the feed trays back into the Feed cylinder. Place the data logger device, 5ml FTG and SD broth onto a metal test tube rack and place it on top of the first tray.
    3. Place the red silicone cap to seal the cylinder and put the red clamp onto the cylinder.

4. **Type B Setup for Feed, Bedding and EnviroPACK** using the collared Feed cylinder.
    1. Refer to the configuration of the feed cylinder in section #3.
    2. 2 reusable mesh bags (Earthwise) containing Biofresh bedding on top of the feed tray.
    3. 1 reusable mesh bag containing EnviroPACKs (as many as needed). Place this on top of the feed tray.
    4. Any other small autoclavable items such as paper bags, fecal collection tubes or pencils can be added.
    5. Place the data logger device, 5ml FTG and SD broth onto a metal test tube rack and place it on top of the first tray.
    6. Place the red silicone cap to seal the cylinder and put the red clamp onto the cylinder.

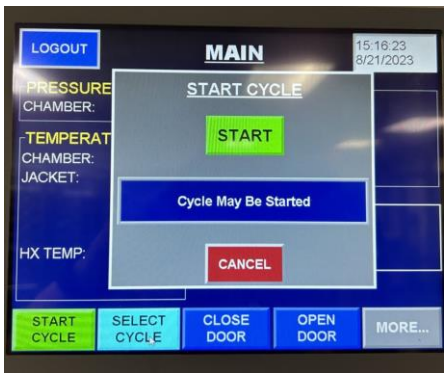
C. Sealing the cylinders with mylar film or red silicone cap




D. Autoclaving the cylinders.

1. Carefully move the prepped cylinder to an autoclavable table on wheels using the hydraulic lift.
2. Roll the cylinder into the clean side cage wash for autoclaving in the bulk walk-in MSB autoclave. Select open door to the autoclave.
3. Place the cylinder into the autoclave. Place a second high temperature data logger probe next to the cylinder on the surface of the table. Select close the autoclave door. Select cycle 07 GB Cages.

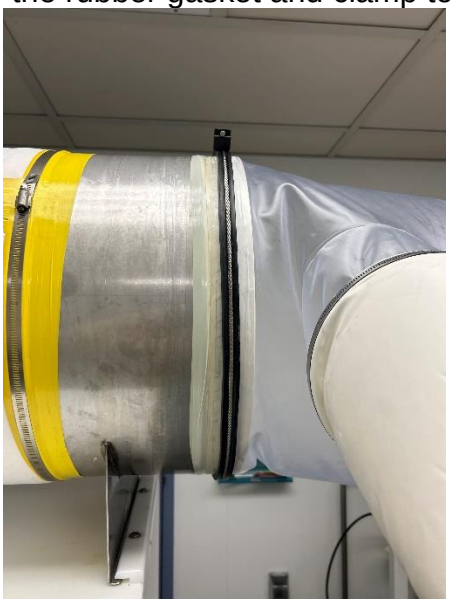

4. Start the cycle.

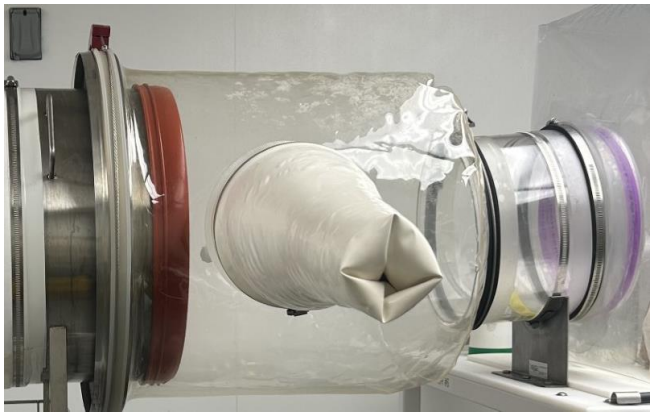


5. When the autoclave cycle is complete, remove the data logger that was placed next to the cylinder. Download the information to ensure the autoclave temperature reaches a minimum of 121^{o}C for 40-minute sterilization time. Once the load is cleared it can be recorded and used for an isolator.

E. Attaching the transfer sleeve to the cylinder.

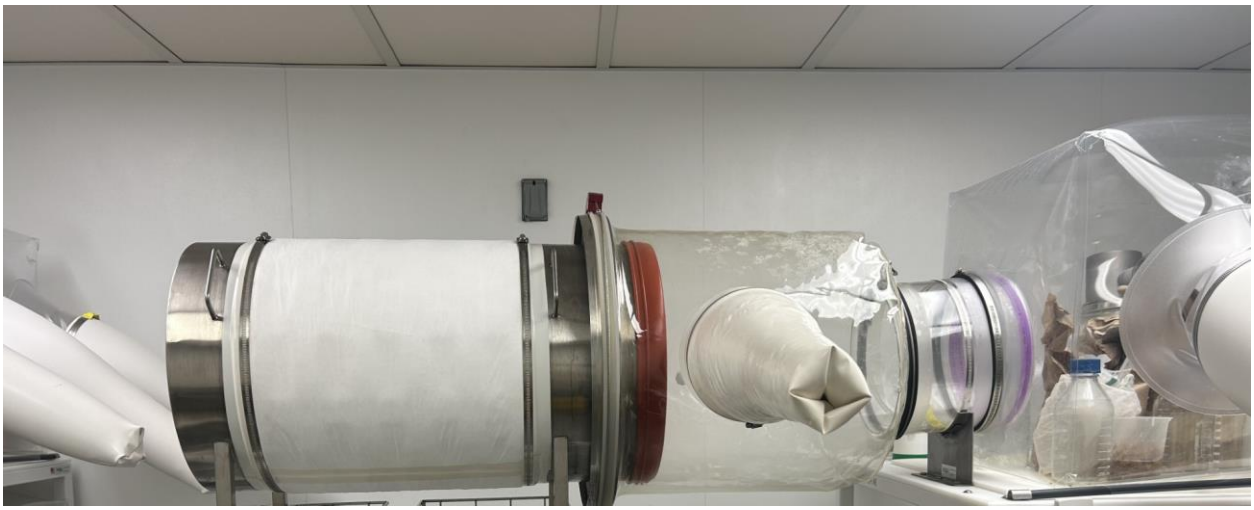
1. Once the cylinder has been cleared with a datalogger the supplies in the cylinder can be transferred into the isolator using the transfer sleeve.
2. Attach the transfer sleeve to the size of the cylinder.
3. Gently wipe off any debris using paper towels from the mylar or red cap.
4. Put on a chemical respirator before hand-spraying with Clidox in the next steps.
5. Spray the mylar or red cap with sterilant and about 2” around the cylinder.
6. Spray the inside of the transfer sleeve along with its nipples with sterilant.
7. Secure the transfer sleeve to the cylinder. There should be about two inches of the transfer sleeve on the cylinder edge. Make sure the seam of the transfer sleeve is either at 12 o clock or 6 o clock.
8. Use the rubber gasket and clamp to securely transfer the sleeve to the cylinder.





9. Attach the transfer sleeve to the isolator port.

10. Open the port cap and wipe any debris with a paper towel.
11. Spray the inside of the port with sterilant.
12. Place the black gasket and port patent clamp around the transfer sleeve in the middle of the sleeve.
13. Spray the outside of the port with sterilant.
14. Pull the end of the transfer sleeve over the end of the port making sure there are about 2" of transfer sleeve on the port.
15. Pull the rubber gasket onto the port over the transfer sleeve. Make sure it is securely on the port 1 cm away from the port edge.
16. Bring the patent clamp onto the gasket and clamp down in place.
17. This will secure the transfer sleeve to the isolator port.
18. Using the atomizer with the extender, fog the inside of the transfer sleeve with sterilant. Refer to SOP # *4.06 Using the Atomizer* to prepare the atomizer for use. Set the timer to 20 minutes and wait for the 20-minute contact time.
19. The transfer sleeve glove should stay inflated during the 20-minute contact time. If the glove deflates then that indicates a leak in the connection and procedure should be aborted.



20. Securely attach one rubber stopper into one nipple of the transfer sleeve. Spray the smaller side of the rubber stopper with sterilant before placing it in the nipple. Place the rubber stopper in the nipple and secure with vinyl tape.
21. If there is a 2nd nipple, spray the 2nd rubber stopper with sterilant so it is ready to be used for the 2nd nipple.