



TITLE: Validation of Cylinders

SOP Category: Gnotobiotic

CMR SOP #: 4.12

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Effective Date: 10/15/24

Approval: *LaTisha V. Moody, DVM, DACLAM*

Revisions: 4/1/24, 9/29/24

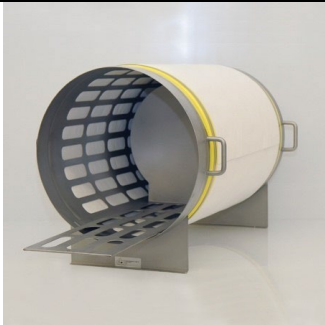

SCOPE:

This document describes the procedures to be followed when validating supply cylinders for flexible-film isolators. This SOP applies to all Animal Care Staff (ACS), ACS Supervisors (ACSS), Veterinary Staff (VS), and Research Staff members (RS) at the Rutgers University facilities.

OBJECTIVE:

The objective is to validate the cylinder for entering supplies into the flexible-film isolator. The purpose of using a cylinder is to pack them with heat-resistant supplies to be placed inside the flexible-film isolator to perform routine husbandry of germ-free (GF) mice.

MATERIALS:

Supply Economy sterilizing cylinder without supply tray 12" or 18"	Feed sterilizing cylinder with collar 18" diameter
	

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PROCEDURES:

Validate Dry Supplies

1. The cylinder wrapped with filter paper is only suitable for 30 uses. After reaching this limit, the cylinder must be re-wrapped.
2. Every item must be placed inside the cylinder to allow autoclave steam penetration. Each tray may be filled slightly above the top of the tray but not overfilled.
3. Empty water bottles and fecal collection tubes should remain uncapped.
4. For the supply cylinder, place 9 clean empty shoe box cages, 9 wire bars, 9 slant water bottles with tops. You can also place rubber bands if necessary. 12"x13" Earthwise mesh bags need to be filled with bedding, Enviro-Pak and paper towel (Wypall)
5. For the feed cylinder, place one layer of feed in each clean tray. 1 bag of gnotobiotic diet should fill all layers inside the feed cylinder. Place the 1st data logger inside of cylinder on any tray inside the cylinder. The best location is to place it into the middle of the cylinder in the most challenging location.
6. Place one tube of Fluid Thioglycolate (FTG) and Sabouraud Dextrose Broth (SD) media. Place the broth media tubes onto an autoclavable test tube rack and ensure tube caps are tightly sealed.

Validate Liquid Supplies

1. The cylinder wrapped with filter paper is only suitable for 30 uses. After reaching this limit, the cylinder must be re-wrapped.
2. Fill 10-12 glass 1L bottles with facility RO hyperchlorinated water according to *SOP # 4.16 Prepping & Sterilizing Water Bottles*.
3. Place the tightly sealed water bottles into the economy supply cylinder.
4. Place one tube of FTG and SD broth media into the cylinder onto the tray.
5. Place the 1st data logger onto the tray inside the cylinder.

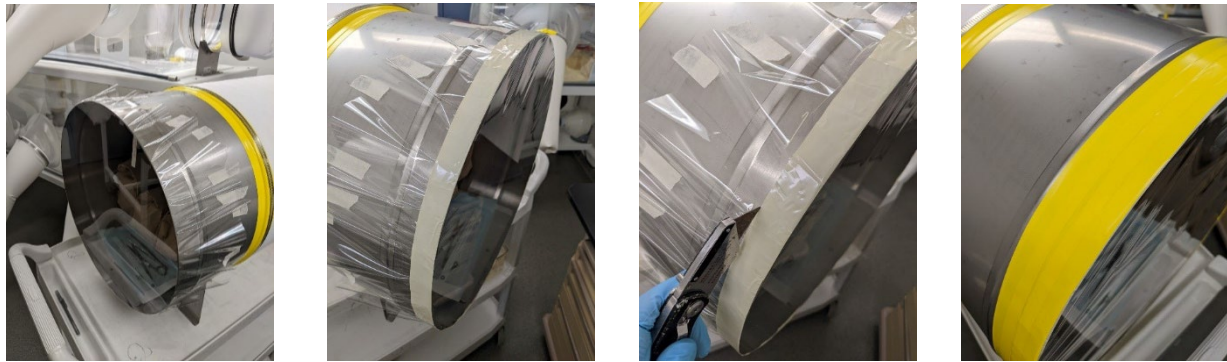
Seal the Cylinder

1. Once the cylinder is loaded with supplies, proceed to seal the cylinder using either clear mylar film & vinyl tape or using the red silicon cap (vendor: NKP isotec) according to *SOP # 4.19 Prepping, Autoclaving Cylinders & Attaching Transfer Sleeve*.

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2. The 2nd data logger must be placed next to the cylinder on an autoclavable safe cart.
3. Place a strip of autoclavable tape onto the metal surface of the cylinder and put the date it was autoclaved. Also write the number of autoclaved uses onto the tape.



Autoclave the Cylinder to Validate

1. For validation purposes, the Omega high temperature datalogger must be placed inside and outside of the load. For secondary backup validation purposes, a biological indicator (BI) may be placed inside and/or outside the cylinder to ensure all contents will be sterilized in the form of liquid suspensions (Prospore ampules) or spore strips (Crosstex).
2. Open the door to the MSB bulk walk-in autoclave which is in the MSB clean side cage wash area.
3. Roll the cart with the cylinder inside the autoclave.
4. Seal the autoclave door.
5. Using green arrows, choose "Cycle 07 GB-cages" for Dry Cycles and click "Select cycle." "Select "Next" and select "Start cycle."
6. Using green arrows, choose "Cycle 08 GB-liquid" for Liquid Cycles and click "Select cycle." "Select "Next" and select "Start cycle."

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7. When the autoclave cycle is complete, carefully remove the 2nd data logger placed next to the cylinder. Download the information from the data logger according to *SOP # 4.32 How to Use the Data Logger*.
8. The data logger must reach a minimum temperature of 121°C for 40 minute sterilization time for the dry cycle or 121°C for 45 minute sterilization time for the liquid cycle. If the appropriate temperature is not reached for the appropriate amount of sterilization time, the cylinder validation fails, and the load must not be used.
9. Record the load information and date loaded on the Autoclave Run Log and the visual clearance signage in the room.