RUTGERS UNIVERSITY Office for Research

CMR STANDARD OPERATING PROCEDURE

TITLE: Unpacking Germ-free Mouse Imports		
SOP Category: Gnotobiotic	**CMR SOP #: 4.28**	**Page: 1 of 4**
Effective Date: 7/9/24	**Approval: LaTisha V. Moody, DVM, DACLAM**	
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SCOPE:

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

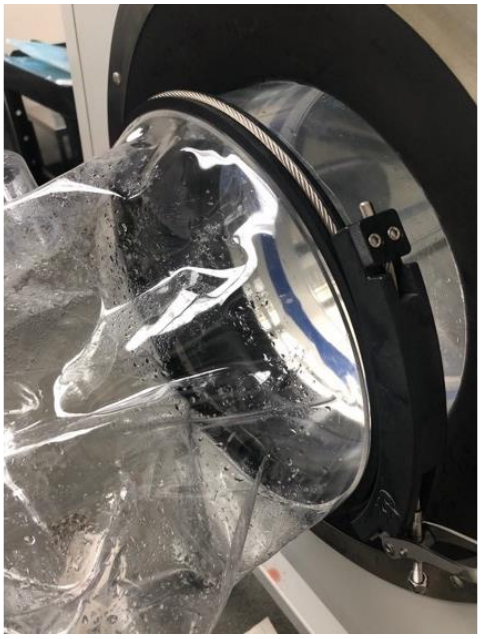
OBJECTIVE:

The objective is to describe the procedure for unpacking imported germ-free (GF) mice and bringing them into the gnotobiotic facility. This procedure requires two trained gnotobiotic staff members.

PROCEDURES:

1. The receiving PI will place an order for germ-free (GF) mice through iLab import request and coordinate with the gnotobiotic core to receive the mice.
2. All GF import unpacking will be performed in a sterilized biosafety cabinet (BSC) through the port in MSB room A685D.
3. Sterilize the BSC including the inner port using Clidox according to the *CMR SOP #4.22 Sterilization of the BSC Surfaces and Gloves*.
4. Obtain the necessary items needed for receiving new GF animals and bring them into the BSC which is described in *CMR SOP #4.04 Gnotobiotic Facility Operations Allentown SPP Caging*.
    a. Sterile wrapped SPP cage containing 2 fecal collection tubes
    b. HEPA filter & igloo if single housed or breeder mice are imported
    c. Sterile food pouches
    d. Sterile water bottle
    e. Long sterile forceps
5. Operator and assistant will don the appropriate PPE including a chemical respirator.
6. Check the shipper for damage before proceeding to the next steps. Check the condition of the animals inside the shipper and document any sick or dead animals. Alert the gnotobiotic manager/supervisor for any sick or dead animals found at the time of accepting GF import.
7. The non-sterile assistant will place the shipper onto a table that is level with the outer port of the Allentown BSC.

   a. Note: the Taconic GF shipper containers can accommodate up to 3 cages of mice with up to 5 mice per cage
8. Spray the Allentown BSC transfer port with sterilant Clidox and scrub surface down using sterile paper towels to clean.
9. Spray the germ free (GF) shipper transfer sleeve including the inside of the sleeve with sterilant 1:3:1: Clidox.
10. Spray the outer port of the BSC with Clidox and attach shipper sleeve to the port.
11. Attach shipper transfer sleeve to outer port of BSC using a patent clamp.



12. Fog the interior port and connected transfer sleeves liberally spray the stopper with Clidox and place the stopper in the nipple to seal.
13. Wait 1 hour contact time.

14. The sterile operator will take off the inner port cap inside the BSC.

15. The non-sterile assistant will remove the tape around the partition of the shipper and turn the partition inside of the shipper horizontally. The sterile operator will pull the partition out into the BSC.
16. The assistant will manipulate the cages through the sleeve towards the operator.
17. The sterile operator will bring cages into the BSC.
18. All newly arriving GF animals will undergo sterility testing to ensure GF status according to *CMR SOP #4.26 Verification of Sterility for GF mice imports*. Animals cannot be used by the investigators until germ-free status is confirmed.
19. Collect 2-3 fresh fecal pellets directly from the anus of each cage will be obtained during transfer from shipper to SPP cages. One fecal pellet(s) will be used for molecular testing (e.g. PCR) by an external laboratory (e.g. Idexx or MRF) and one of fecal pellet(s) will be used for independent testing by in-house microbiological aerobic and anaerobic culture testing.
20. The sterile operator will place the mice into new cages, add new food, water, and the intra-cage HEPA filter. Seal the cage and place feces and swab samples on top of the cage.
21. Remove the mouse cage with the samples on top by placing the mouse cage as close to the front partially obstructing the grill with the HEPA filter pointed towards the cabinet rear.

22. Remove the mouse cage with the samples, put the new RFID cage card onto the cage card holder.
23. Perform the cage leak check according to the CMR SOP *#4.04 Gnotobiotic Facility Operations Allentown SPP Caging***.** Wipe off excess Clidox if necessary and place the cage onto the rack. Label the cage “Testing in Progress.”
24. Remove the water bottle and other items you want to remove from the BSC.
25. Replace the inner port on the BSC before disconnecting the shipper sleeve.
26. Clean the BSC and transfer port and any other surfaces that were exposed to sterilant according to the *CMR SOP #4.21 Cleaning the BSC surfaces and glove*