RUTGERS UNIVERSITY Office for Research

CMR STANDARD OPERATING PROCEDURE

TITLE: Gnotobiotic Facility Operation SPP Caging		
SOP Category: Gnotobiotic	**CMR SOP #: 4.04**	**Page: 1 of 10**
Effective Date: 9/17/24	**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 to all trained Gnotobiotic Veterinary Staff, and Research Staff members only (RS) at the Rutgers Gnotobiotic University facilities.

OBJECTIVE:

The objective is to operate equipment safely and securely using the Allentown individually sealed positive pressure (SPP) Sentry caging system.

TERMINOLOGY:

- **SPP cage** – Sealed Positive Pressure gnotobiotic mouse cage manufactured by Allentown, Inc.

- **Sterile Operator-Assistant Two-Person System**
  1. There will be one sterile technician, the operator, who will don sterile gloves, gown, and perform sterile procedures only. Touching only sterile materials that will touch the inside parts of the Allentown Sealed Positive Pressure (SPP) cages.
  2. The second technician, the assistant, will wear non-sterile gloves and perform all non-sterile procedures by only touching non-sterile materials and the outside of the SPP cages.

- **Primary Operator System –** one technician acting as both the sterile operator and non-sterile technician by use of Honeywell glove attachment on wheel trolley (Allentown)

- **Transfer Chamber (TC) –** a chamber that is connected to the biosafety cabinet (BSC) that facilitates transfer of IVC cages

PROCEDURES:

A. *Cage Change Frequency & Space Requirements*

1. Germ-free (GF) animals and gnotobiotic animals produce none to little odor due to few ammonia-converting urease-bacteria produced in their feces and urine. According to the 8th edition of the *Guide for the Care and Use of Laboratory Animals*, some types of cages and housing systems may require less frequent cleaning or disinfection such as low animal density and gnotobiotic conditions. A visual method may be used to evaluate cage wetness to determine frequency of changing depending on cage density but typically are changed every 2-4 weeks.
2. SPP cages will be spot changed when visibly soiled or wet.
3. Up to 5 adult mice can be housed in the SPP cages.
4. Criteria for spot cage-change in gnotobiotic SPP cages:
   - BioFresh bedding is ≥75% wet
   - BioFresh bedding has ≥ 50% feces covering the surface
   - When in-cage HEPA filter is due to be changed (according to the manufacturer, in-cage HEPAs must be discarded after 16 weeks of use or 12 autoclave cycles)
   - Any flooded cage must be immediately changed.

DRAPE ONLY TECHNIQUES

***B. Using 2 Person* Sterile Operator/Assistant System (Drape only technique)**

1. This method does not require a chemical sterilant or respirator.
2. This procedure is used for moving animals from flexible-film isolators to IVC cages.
3. The non-sterile assistant will open the sterile double wrapped cage and unwrap the first layer drape to use as the sterile surface.
3. Primary operator dons surgical PPE attire (e.g. 1 pair of sterile gloves, 1 pair of sterile Tyvek sleeves).
4. The sterile operator removes the sterile cage lid and wire bar and places it to the side on the sterile drape.
5. The assistant will carefully unroll the bag and gently dump animals into the cage without the dirty outer paper bag touching the sterile cage.
6. The assistant unwraps the outer HEPA sterilization pouch and the sterile operator grabs the inner sterilization pouch. Install the in-cage HEPA filter on the grommet in the back of the clean SPP cage. Use a slight twisting push motion and hold the cage stable by placing the other hand on the outside of the back of the cage.
7. The assistant unwraps the outer layer of the sterilized food pouch and operator grabs the inner sterile pouch. The operator opens the food pouch & pours the feed into the hopper.
8. The assistant will open the 1 L sterilized water under the BSC and the primary operator holds open the cage bottle. The assistant will carefully pour the water into the bottles and the operator closes the bottle with sipper tubes. The operator will replace water bottles into the cage hopper and close the cage lid and secure the lid.

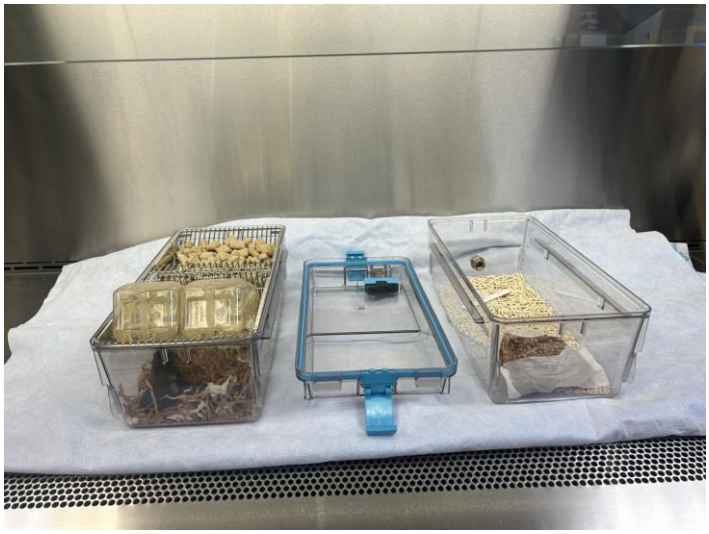
9. The cage will be returned to the rack after testing the seal at the leak testing station.

C. *Single Operator System (Drape only technique)*

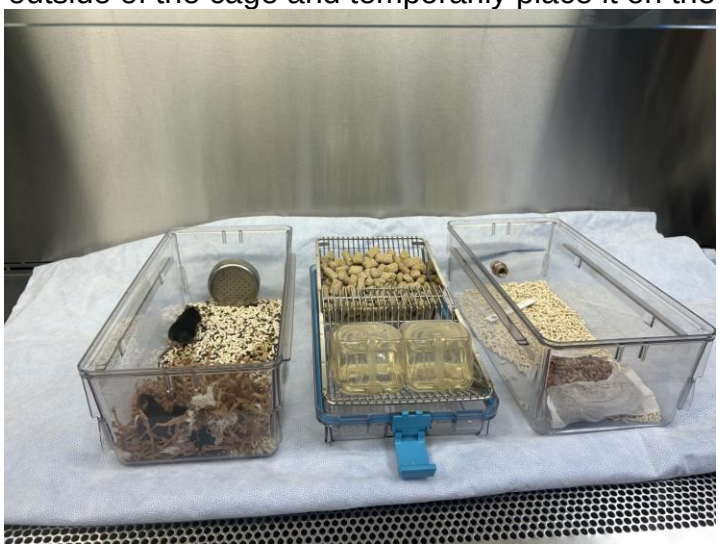
1. This method does not require a chemical sterilant or respirator. This method is an alternative way to change SPP cages when only a single operator is available. The single operator will utilize both sterile and non-sterile PPE to complete activities.
2. Wearing regular gloves, place a sterilized single-wrapped SPP cage bottom on the work surface under the BSC.

3. Remove the autoclaved tape, unwrap the SPP cage carefully, touch only the outside of the wrap (dark side) while unfolding it, making sure to not block the air flow.
4. Remove the soiled SSP cage from the rack and place it on the sterile drape approximately 8 to 10 inches to the left of the sterile SPP cage bottom.

5. Unlatch the lid on both sides of the soiled cage and snap the clamps into the hold open position. Remove the lid from the cage, invert it and place it with the inside facing up on the sterile drape. Take care not to touch the rim or the inside of the lid.

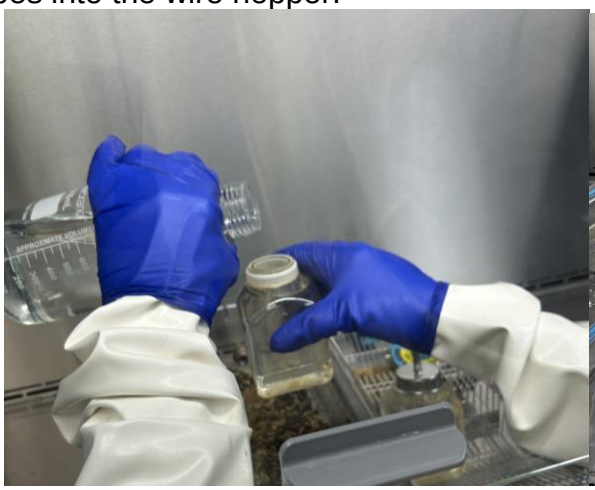


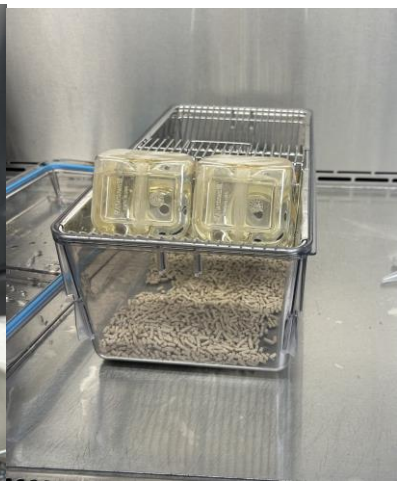
6. Remove a bag of feed from external autoclaved pouch and drop it on the sterile drape.
7. Loosen the cap from the sterile water bottle and place the bottle next to the soiled cage on the BSC.
8. Remove regular gloves to don sterile gloves and sterile sleeves.
9. Lift the wire bar lid off the soiled SPP cage with one sterile hand taking care not to touch the outside of the cage and temporarily place it on the inverted lid



10. Remove the in-cage HEPA filter from the soiled cage and with the other sterile hand using a slight twisting motion. Install the in-cage HEPA filter on the grommet in the back of the clean SPP cage. Use a slight twisting push motion and hold the cage stable by placing the other hand on the outside of the back of the cage. Make sure to not touch the cage rim with the sleeve of the gown.
11. Top off feed by opening the sterile pouch and dropping food into the wire bars.
12. Top off water last. Using sterile gloves open the 2 sipper tubes and place them on the wire hopper. By using your sterile left hand to grab the glass water bottle and hold the cage water bottle with the sterile right hand. Carefully fill the cage water bottles ensuring the non-sterile surface doesn't touch the sterile cage

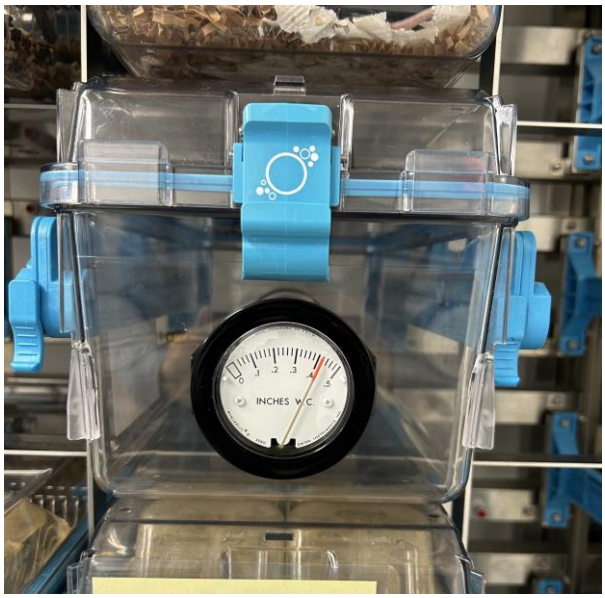
bottles. Using the sterile right hand only, replace both cage bottles and sipper tubes into the wire hopper.





13. Place the SPP cage lid on the clean cage and the cage is sealed by fastening the clamps.
14. The SPP cage is tested in the seal testing station and the cage slot on the rack is tested using the test cage.

Seal testing Station



Testing cage slot on the Rack

15. The cage is placed into the tested cage slot making sure that it is fully inserted, and the yellow cage-disengaged indicator disappears. Refer to above section “Leak Testing.”
16. A cage card holder is attached to the cage and the cage card is inserted.
17. Place the cage back in the appropriate spot on the rack.

FULL ASEPTIC TECHNIQUES

D. *Entering an SPP Cage Containing Animals into the Biosafety Cabinet*

1. Take the cage off the Allentown rack by pressing down blue lever to the right. The yellow circle will be visible which indicates the cage is not properly hooked up to the SPP caging system.

2. DO NOT let the cage be in its sealed position out of the rack for longer than 45-60 minutes as animals will suffocate.
3. Remove the cage from the rack and place inside disinfected TC according to *SOP # 4.22 Sterilization of BSC surfaces and gloves*.
4. If using full aseptic technique with Clidox on BSC surfaces or Honeywell glove system, spray all sides of cage with sterilant using the turn table in the TC to spray the entire cage including the bottom of the cage. Don the half face chemical respirator when spraying with 1:3:1 Clidox.
6. Set timer for 15 minutes and wait for the cage to be sterilized.
7. The inside surfaces of the BSC are sterilized prior following *SOP # 4.22 Sterilization of BSC surfaces and gloves*. The sterile operator dons sterile attire.
8. Operator places hands in hood under the sash making sure the bottom of arm is not resting on the hood surface. Open the door to TC from inside the hood and place cage on hood surface making sure the airports are facing the back of the hood. Close door to TC.
9. Unlock cage lid by pushing the top part of blue latch down on both ends of the lid. Take lid off and push blue latches up and lock into position on top of lid. Place the lid, upside down, to the side of the cage. Take wire bar off cage and place on top of lid.
10. Manipulate animals or perform husbandry as needed.

E. Don Personal Protection Equipment (PPE) when not using Honeywell gloves

1. Proper PPE for the sterile operator is required which includes surgical face mask, hair bonnet, two pairs of sterile gloves, and sterile sleeves whenever work is

being done in the biosafety cabinet (BSC). Sterile PPE must be done in this order:

2. Open sterile surgical Tyvek sleeve package.
3. Open sterile glove package.
4. Don on gloves by opening sterile package, place dominant hand in glove making sure not to touch the outside of the glove with bare hand, use gloved dominant hand to put on opposite glove. Repeat with a second pair of gloves.
5. Put sterile sleeves on using sterile gloves.

F. Entering wrapped autoclaved SPP cages, changing the food & water in the BSC with Honeywell glove Attachment - using Single Primary Operator System

1. This procedure will utilize a single Operator.
2. Ensure the BSC & TC is sterilized prior to entering supplies.
3. Primary operator don's non-sterile gloves initially spray down surfaces with Clidox.
   a. Don chemical half face respirator.
   b. Sterilize the inside of the TC and BSC including the Honeywell gloves on wheels and set timer for 15-minute contact time. Spray in and place cage(s) to be changed, water bottle(s) and swabs into the TC and set timer for 15-minute contact time.
4. Turn on BSC, it will beep for up to 3 minutes. Wait up to 3-5 minutes until beeping stops before use of BSC.
5. Spray non-sterile gloved hands with sterilant and place gloved inside of the Honeywell gloves. And place another regular larger glove on top of the

Honeywell glove. Spray with sterilant and lay them in the hood and attach Honeywell gloves to the wheel apparatus.

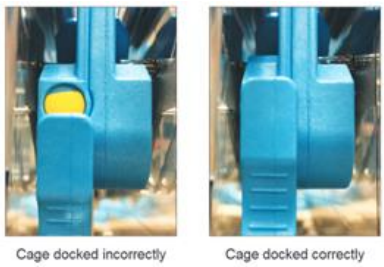
6. Spray sides of no more than 2 animal cages, glass 1L water bottle per cage, 2 glass media tubes per cage with sterilant using the turn table to spray the entire cage including the bottom of the cage.
7. Close the door to TC and ensure the handle is securely in its locked position.
8. Prepare your caging and supplies:
    a. The operator will don non-sterile gloves to unwrap the outer wrappings of:
        i. The sterile cages (4-6 cages can fit into the 6ft BSC)
        ii. Sterilized pouch of food (need 1 pouch per cage)
        iii. Sterile surgical gloves & sterile sleeves
    b. The operator will place the sterile items into the BSC (note: make sure to push the Honeywell gloves out of the way).
9. Unwrap your sterile supplies inside of the BSC such as the new cage. Bundle up the drapes and put them to the side to discard later. Do not block the BSC's front or rear air intake grills, this will interfere with the hood's air curtain and risk contaminated air entering the sterile BSC.
10. Open the TC to bring in the supplies after they have had a full 15-minute Clidox contact time.
11. Open the mouse cage under the hood to allow the mice to breathe.
12. Before changing the mouse cage inside of the BSC, the operator will remove gloved hands from the Honeywell gloves and spray in the next 2 mouse cages, water bottles and 2 swabs into the TC. Spray with Clidox 1:3:1 for a 15-minute contact time
13. Put gloved hands back into the Honeywell gloves and continue the cage change and sample collection which is described in *CMR SOP # 4.23 Routine Sterility Testing for GF mice Colony*.
14. To retrieve samples and remove supplies from BSC:
    a. Collect your samples and place them on top of the cage.
    b. Push Honeywell gloves to the left side and move items to be removed onto the right side of the BSC.
    c. Remove the mouse cage first. Using the Honeywell gloves, place the mouse cage as close to the front partially obstructing the grill with the HEPA filter pointed towards the cabinet rear.

d. Remove hands from the Honeywell gloves and carefully remove the mouse cage, replace cage card, leak check the cage (see section F: ***Testing Cage Seal*** *below****),*** *wipe off excess Clidox if applicable and place cage back onto the rack.*
e. Remove the water bottle in a similar manner, and other items you want to remove.
f. NEVER let the sterile Honeywell gloves cross over the grill****

15. To clean up, turn off the BSC light and blower. Wipe down debris with Clidox and paper towels, spray sterile water on all surfaces including the Honeywell gloves according to *CMR SOP # 4.21 Cleaning the BSC surfaces and Gloves*.

G. Transferring a SPP cage out of the hood and put onto SPP rack

1. Securely lock the cage lid to the cage bottom with the latches.
2. Take the cage out of the BSC.
3. Test the seal on the cage and dock the cage correctly on the rack securely once leak testing is completed.



H. Testing the Cage Seal

1. Place the cage in Seal Testing Station (STS) to ensure the cage is properly sealed.

2. Turn on STS using the switch on the side of the station.
3. Press the blue on/off button in front of the station.
4. Press blue lever on ride side and place cage in blue slots so it slides in smoothly.
5. The yellow circle will be covered by a blue latch when the cage is properly placed.
6. Press the start button on top of the station.
7. The screen will show "test in progress."
8. If the test passes, the word "pass" will be on the display along with the green light illuminated. The green light indicates the test has passed.
9. If the test fails, the word "fail" will be on the display along with the red light illuminated. The red light indicates the test has failed. To test again, press the "reset" button on top of the station and press the test button again.
10. If test fails, retest. If the test fails again place cage back in the hood, securely lock lid, and retest.
11. If test fails a third time:
    a. Spray the cage bottom. Put cage on the sterile blue drape and open the cage lid with gloved hands. With sterile gloved hands, adjust wire bar and water bottles or anything out of place. Replace the lid and seal the cage to retest.
12. Record that test has failed and date on the cage card.

I. Animals Found Dead Inside SPP cages:

1. Any animal(s) found dead will be left inside the cage for 48 hours until a trained gnotobiotic technician can remove the carcass.
2. Scan the QR code on the cage and an emailed report will be sent to the principal investigator and lab contact. The person scanning the QR code, should click the “Notify CMR vet staff” button so the gnotobiotic staff receives the notification.
3. Animal will be placed in a sterile mouse carcass bag or biohazard bag and labeled.

   a. Note: GF animals lack any microorganisms, there is minimal risk of odoriferous putrefactive changes and autolysis of the intestinal area by digestive enzymes and release of ammonia associated with decay. This is standard practice in germ-free facilities. GF animals typically undergo drying and mummification only.
4. If applicable, a necropsy will be performed on the animal by a trained technician or veterinarian.