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**Effective Date: March 8, 2019**

**Supersede: NA**

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#### PURPOSE

To outline a schedule, from study start to end, for Germ-Free models, in particular how mice should be handled to maintain a Germ-Free state.

RESPONSIBILITY: Scientist/Technician

#### PROCEDURES:

##### **Study Preparation:**

1. Gather any study supplies into the BioBubble so that they can be Halo fogged (refer to SOP#Lab29 HALO FOGGER 110 OPERATION) prior to the arrival of mice. These include, but not limited to:
  - a. Fecal collection tubes (labeled), caging, food hoppers, water bottles, extruded autoclavable diet, corncob or diamond dry bedding (per protocol), blue pads, scale, clear solo cups, stainless steel cup, copy of the study protocol, biohazard boxes, and PPE
2. Supplies should be sufficient to last the duration of the study, within practicality, to minimize the number of extra supplies that must be brought into the BioBubble once the study has begun.
3. Expose every surface in order to be sterilized by the Halo fogger (i.e.- tip biohazard boxes on their edge, open any bottles or bins, set cages on shelf, etc.).
4. Position the Halo fogger in a corner of the BioBubble, facing the center. Turn off the H.E.P.A. filters (switch is located on the top left back of the machine).
5. After the BioBubble has been fogged and it is safe to again enter the BioBubble.
  - a. Reposition the Halo fogger to the opposite corner from its original position.
  - b. Move any objects so as to expose any surfaces that may not have been sterilized by the first fogging.
6. After fogging twice, don appropriate PPE (i.e.- Tyvek suit, hairnet, respirator, goggles, gloves, and shoe covers) to remove the Halo fogger and prepare the BioBubble for the receipt of mice.

### **Receipt of Mice:**

1. Receive mice received and transport to West Wing according to SOP #Lab02 Receipt of Animals.
2. Two scientists will be needed to transfer mice into the positive pressure BioBubble—one inside and the other outside of the BioBubble.
3. Transfer mice into the BioBubble following the below steps. Great care should be taken to ensure the mice and BioBubble remain germ-free because this is the biggest opportunity for contamination:
  - a. The scientist inside the BioBubble steps just inside the threshold to don all appropriate PPE and spray their gloves, sleeves, and the stainless-steel table with Peridox®.
  - b. The scientist outside the BioBubble who is passing the box into the BioBubble via the port will don appropriate PPE (disposable lab coat, N95 respirator, gloves goggles and shoe covers) and will then remove the outer cardboard packaging to expose the “middle” plastic packaging, which will contain one to three mouse cages as well as a H.E.P.A filter taped on the end.
  - c. Spray this plastic packaging with Peridox® and align with the 18” port cut into the front of the BioBubble using a stainless-steel table.
  - d. The scientist on the outside of the BioBubble will slowly feed the plastic packaging through the port, so that the scientist inside may cut around the H.E.P.A filter and remove the mouse cages.
  - e. Transfer the mice to appropriate Innovive caging and provided with food, water, and Diamond Dry or corncob bedding per study protocol.
  - f. Handle mice using sterile, autoclavable forceps when possible to minimize the chance of contamination.
  - g. Use the forceps to grasp the extra skin between the mouse’s shoulder blades, as though you were trying to scruff the mouse.
4. Once the mice have been transferred to cages and any study activities performed (i.e.- fecal collections, microbial challenge, or fecal collection) remove PPE and hang on a rack near the BioBubble entrance (within reach and preferably on the opposite side as the mice) for reuse.
  - a. Only reuse Tyvek suits, shoe covers, and goggles. Wear new gloves and respirators from the stock of Halo fogged supplies within the BioBubble each time the BioBubble is entered.

### **Fecal Collection & Treatment:**

1. Don all appropriate PPE (i.e.- Tyvek suit, hairnet, respirator, goggles, gloves, and shoe covers) upon entering the BioBubble and spray any work surfaces with a sporicidal (ex. Peridox<sup>®</sup>) prior to use.
2. Collect feces according to the study protocol (i.e.- clean cage, cage collection, number of pellets, etc.).
3. Spray gloves with a sporicidal (ex. Peridox<sup>®</sup>), change oral gavage needles, and replace the clear solo cup when switching between groups during fecal collections or treatment.
4. Administer treatment alongside fecal collections.
5. When possible, perform fecal collections and treatments at approximately the same time each day, unless otherwise stated in the study protocol.
6. Upon leaving, sweep the BioBubble and wipe down the tabletop using a sporicidal (ex. Peridox<sup>®</sup>).
7. If any materials must be brought into the BioBubble in order to complete study activities they spray them extensively with a sporicidal (ex. Peridox<sup>®</sup>) upon entering allowing a 3 minute contact time.

### **Study End:**

1. On the last day of the study, euthanize the mice according to the Animal Use Protocol. Dispose of any remaining bedding, caging, or food into a biohazard box.
  - a. Perform required tissue harvests at this time.
2. Pour water into a bucket and leave them in the BioBubble to be Halo fogged.
3. Expose every surface in order to be sterilized by the Halo fogger (i.e. tip biohazard boxes on their edge, open any bottles or bins, etc.).
4. Position the Halo fogger in a corner of the BioBubble, facing the center. Turn off the H.E.P.A. filters (switch is located on the top left back of the machine) and halo fog the BioBubble.
5. After the BioBubble has been fogged and it is safe to again enter the BioBubble.
  - a. Reposition the Halo fogger to the opposite corner from its original position.
  - b. Move any objects so as to expose any surfaces that may not have been sterilized by the first fogging.
6. After fogging twice, prepare the BioBubble for the next study (i.e.- biohazard boxes removed, new supplies brought in, etc.).