

## **FLSC Standard Operating Procedures for Husbandry of Immunocompromised Mice**

### **Purpose**

Athymic nude and other immunocompromised mice are at greater risk than conventional mice for the development of infectious disease. Frequently, infectious pathogens are transferred to immunocompromised mice by contaminated equipment or exposure to contaminated environments. The procedures described here are implemented to minimize the risk of infectious disease in immunocompromised mice.

### **General Procedures**

1. All equipment and caging is sterilized prior to using with the immunocompromised mice.
2. Prepare the laminal flow hood/Biosafety Cabinet 24 hours prior to manipulating the mice.
  - a. Spray with disinfectant and wipe down the interior surfaces including the back and sides of the cabinet.
  - b. Spray the interior of the hood with 70% ethanol with the blower on.
3. The hood blower is left on at all times.
4. The light is turned off when the hood is not in use.
5. The cabinet sash is closed leaving a 3 to 4 inch opening. Closing the sash completely turns off the blower.
6. All individuals handling the nude mice must wear a steam sterilized gown, mask, hair bonnet, and sterile gloves. Optimally, two individuals are present during the handling of the mice, one that is designated as the animal handler and maintains sterility, while the other handles the non-sterile items and opens the sterile materials for the handler.
7. It is required that persons reaching into the hood wear a clean long-sleeved gown, lab coat or smock, mask and hair bonnet and disposable gloves to prevent contamination of the sterile interior.
8. Aseptic technique should be used at all times when handling the nude mice. Any objects or surfaces that will come in contact with the mice should have been sterilized by steam, UV radiation, or contact with 70% ethanol for at least 30 minutes.
9. All animals will be checked daily, including weekends and holidays.
10. Examine the mice by viewing them through the cage.

### **Unpacking Mouse Shipments**

1. Prepare laminar flow hood as previously described. Animal handlers must be prepped, as described above.
2. Shipping labels, health reports, cable ties and animal information are removed and recorded by the non-sterile assistant prior to placing the shipping container into the hood.
3. Shipping containers are sprayed with a chlorine dioxide solution before they are placed in the hood. This includes spraying the bottom, sides and top of the container.
4. The designated animal handler opens the sterile food and water under the hood.
5. The non-sterile person opens shipping box under the hood. Care must be taken by the non-sterile person to avoid contact with the inner surfaces of the hood and the inside of the shipping box and cage.
6. When using sterile Vent Rack cages:
  - a. The cage is passed aseptically to the animal handler.
  - b. The cage bonnet is removed and placed upside down in the hood.
  - c. The wire bar cage top is placed on the bonnet so that the wire bar cage top rests on the interior surface of the bonnet.
  - d. The cage is lifted with the sterile hands on the inner surface of the cage and transferred to the charging station.
  - e. The animal handler depresses the sipper to charge the sipper assembly with water.
  - f. The cage is removed from the charging station and returned to the hood maintaining the sterility of the cage.
  - g. The animal handler then places the wire bar cage top crosswise and adds food.
  - h. The animal handler removes mice from the shipping box and places them in the cage.

- i. Vent Rack cages are limited to 5 mice per cage.
  - j. The wire bar cage top is placed on the cage.
  - k. The non-sterile person places the bonnet on the cage being careful not to touch the sterile interior side of the bonnet before removing the cage from the hood.
7. When using sterile Innovive® cages:
    - a. The cages, cage tops and feeders are placed in the hood and the outer plastic bags are sprayed with 70% alcohol paying special attention to the twists in the top of the bags.
    - b. A cage and feeder are removed from the plastic bag and the feeder is placed in the cage.
    - c. Orient the cage so that the longer front lip on the cage is facing forward.
    - d. Food is added to the feeder not above the maximum fill line.
    - e. The animal handler removes mice from the shipping box and places them in the cage.
    - f. Innovive® cages allow 6 mice per cage.
    - g. The animal handler removes a sterile cage top from the bag and orients it so that the depression for the water bottle is in the front of the cage.
    - h. Place the cage top on the cage and snap the top securely at all four corners.
    - i. Remove a sterile water bottle from the plastic and peeled away the seal on the lid.
    - j. Place the sterile water into the water bottle depression on the cage top.
  8. The non-sterile person will remove the cage from the hood.
  9. The animal handler should re-glove after each shipping container to avoid possible cross contamination between groups in case the shipping container has been damaged.
  10. Record the container number on the cage card to track origin of animals in case of illness.
  11. Unused water bottles are stored in the plastic bag with the sterile cages for future use.
  12. Unused food is given to FLSC personnel for use in the breeding colony rooms.
  13. Mice will undergo a one to two week acclimation period prior to experimental use.

### **Cage changing**

1. Follow all previous steps for hood preparation, gowning of personnel and cage handling.
2. The designated animal handler should re-glove after every 6-10 cages or if gloves are torn, contaminated or if a cage has ill animals.
3. Any cages with animals appearing sick will be placed at the end of the cage changing order to avoid possible exposure of healthy animals to pathogens or contaminants.
4. Vent Rack cages are changed at 14 days or more often, if deemed necessary.
  - a. Vent Rack cages are sterilized as a complete unit consisting of a bedded cage, environmental enrichment, cage top with feeder and filter bonnet.
  - b. Vent Rack cages will be replaced as a unit at each cage change.
5. Animals housed in the Innovive® caging will require weekly cage changes.
  - a. Innovive® caging products come separately as empty cages, feeders, and cage tops.
  - b. Feeders will be changed every 4 weeks.
  - c. Cage tops will be changed every 8 weeks.
  - d. Cage tops and feeders will be reused unless there is a break in sterility necessitating replacement of the contaminated component.
  - e. Environmental enrichment is autoclaved separately and added to the cages as they are used.
6. If food and/or water must be added between the scheduled cage changes, or dead animals removed, it must be done aseptically under the prepared hood.
7. Sterile water is purchased prepackaged.
  - a. Innovive water bottles are pre-sterilized and are replaced weekly unless acidified.
  - b. Acidified sterile water bottles are changed every other week.
  - c. To acidify Innovive® water bottles, place 0.15 ml of 12M HCl into each bottle through the lid opening using sterile technique
8. In the event an investigator wants sterile water with specific additives or treatments, the preparation and sterilization are the responsibility of the PI.
  - a. Water is acidified by adding 2 drops of 12M HCl to 8 liters of RO water.

- b. Water is sterilized in individual bottles covered with foil.
  - c. Bottle lids are sterilized separately in an autoclave bag.
  - d. Sterilized water in 250 ml glass bottles will require tall bonnets on cages to accommodate the bottle.
  - e. The cages with tall bonnets will be housed on shelf racks and changed on the same schedule as the Innovive® cages.
9. Caging is stored on a covered cart in the animal room or in a container next to the hood.
  10. Any special diets must be provided by the PI. If diets are not sterilized by the manufacturer, the PI is responsible for autoclaving or sterilization of the food.
  11. Gowns are laundered, wrapped and sterilized by FLSC staff. Should an experiment require daily gowning by multiple persons, disposable sterile gowns should be purchased by the PI and stocked in the storage cabinet in the room with the hood.

### **Precautions**

1. The investigator must provide emergency contact information to FLSC including a phone number where the responsible lab member(s) can be reached.
2. Any special housing, food or water requests must be communicated to the FLSC management.
3. The sterile cage should NEVER be opened outside the sterile hood.
4. The sterile cages should not be opened on weekends or holidays without the permission of the investigator or FLSC management.
5. In the event of a problem, the investigator (see the contact list on the door) should be called. Emergencies should also be directed to FLSC management.