STANDARD OPERATING PROCEDURES

DIVISION OF COMPARATIVE MEDICINE
UNIVERSITY OF SOUTH FLORIDA

TITLE: **Isolation Rodent Husbandry and Use**
SCOPE: All Authorized Personnel
RESPONSIBILITY: Facility Manager and Technical Staff
PURPOSE: To Outline the Proper Procedures for Safely Caring for and Using Immune Deficient Mice

I. PURPOSE

1. To outline the proper procedures for safely conducting husbandry and handling of immunodeficient rodents housed in isolation in sterilized microisolator caging.

II. RESPONSIBILITY

1. The Facility Manager is responsible for ensuring:
    a. All animal and research personnel are adequately trained to perform the practices described.
    b. Implementation of the procedures described.
    c. Appropriate personal protective equipment (PPE) is available and used.
    d. Documented training of all personnel with access to isolation housing and use areas.

2. It is the responsibility of all animal and research personnel to read, understand, and follow the procedures outlined below.

3. Access to isolation rodent housing and use is limited to essential personnel, and only those with documented didactic tutorial and in-person training presented to the IACUC.

III. PROCEDURES

III.A. Preparation of the Primary Enclosure

1. **Autoclaved chow** and **acidified or autoclaved drinking water** are provided to rodents in isolation.

2. Microisolators used for isolation rodent housing must be **secondarily covered** prior to transport to isolation, provided with autoclaved chow, and interiors validated as sterile in accordance with SOP 1002 *Monitoring Autoclave Sterilization* with an **Integrator strip** present with each autoclaved load.

3. Acidified drinking water is prepared in accordance with SOP 417 *Preparing Acidified Drinking Water for Rodents*. Autoclaved drinking water is prepared in accordance with SOP 1006 *Autoclave Sterilization.*

4. Racks of bottles filled with acidified drinking water must be validated as sterile in accordance with SOP 1002 with an Integrator strip present with each autoclaved load, and the racks of bottles **covered** prior to transport to isolation**.**

5. Ensure that all caging and water bottles have been properly autoclaved by checking Integrator strips present with each autoclaved load of cages or water bottles. Refer to Monitoring Autoclave Sterilization (SOP 1002), Autoclave Sterilization (SOP 1006), and Monitoring Steam Sterilization of Liquids (SOP 1013) for proper sterilization techniques. If steam exposure indicator has not changed, do not use cages/supplies and notify your supervisor immediately.

6. Whenever covered autoclaved transport racks of microisolators or covered racks of bottles or empty water bottle racks or empty transport racks are delivered to the isolation housing room door, a **two person transfer procedure** should be utilized with:
    a. The interior individual donning PPE in advance in accordance with entry procedures described below,
    b. Keeping the door **closed** while the exterior individual uncovers, then **saturate sprays** the exterior of equipment/supplies with **Oxivir**,
    c. The door is opened, and the sprayed equipment/supplies either transferred to the interior cart, or the cart is rolled over the **antimicrobial adhesive mat** just inside the door,
    d. The door is closed, and the cart, rack, and equipment/supplies **sprayed again** by the interior individual, and allowed to **sit for 5 minutes** inside isolation.
    e. Racks of water bottles are covered with a Tyvek cover when kept within the room.

III.B. Preparation of the Secondary Enclosure

1. Isolation housing and use rooms should be **prepared in advance** of room occupancy with all equipment and supplies required for the study **dedicated** to the isolation room and **decontaminated in advance of use**. An **antimicrobial adhesive mat** is placed just inside the door and contact sheets removed and changed at least daily.

2. Isolation housing rooms are **monitored monthly for opportunistic bacteria** in accordance with SOP 410 *Sentinel Health Surveillance*.

3. When an agent excluded from isolation is detected, importations to the room cease, studies complete to end point, housed inventories are depopulated, and the room and its contents decontaminated by using **vaporized hydrogen peroxide** in accordance with SOP 1016 *Hydrogen Peroxide Vapor Decontamination* and SOP 1162 *Bioquell Z-2 Hydrogen Peroxide Vapor Generator System*.

4. Racks, trolleys, shelving units, carts, and other **portable equipment** (e.g., scales, calipers, anesthesia machines, surgical instruments, clippers, heating pads, tattoo machines, IVIS 100) and **consumable supplies** (e.g., weigh boats, syringes, needles, gauze sponges, blood collection tubes, specimen containers) should be delivered to the isolation room during new room preparation and prior to room

decontamination.

5. Decontaminate the newly prepared isolation room and its contents, and validate efficacy of the decontaminating process in advance of occupancy, using **vaporized hydrogen peroxide** in accordance with SOP 1016 *Hydrogen Peroxide Vapor Decontamination* and SOP 1162 *Bioquell Z-2 Hydrogen Peroxide Vapor Generator System*, and at the discretion of the veterinarian, using environmental sampling for **detection of opportunistic bacteria** in accordance with SOP 410 *Sentinel Rodent Health Surveillance*.

6. Supplies and equipment must be dedicated to isolation housing and use.

7. If additional consumable supplies, equipment, reagents or biologics are needed during study, they must be placed in **nonporous secondary containers** (e.g., Ziploc bags, conical tubes, screw-top containers, autoclave-able bags) and either sterilized by autoclaving or vaporized hydrogen peroxide, or disinfected by saturate spraying with Oxivir TB. Containers are then delivered using a **two person transfer procedure** as described above.

III.C. Personnel Entry

1. Access to isolation rodent housing and use is limited to essential personnel, and only those with **documented didactic tutorial and in-person training** presented to the IACUC.

2. Work in isolation housing and use must precede all other work in any other animal facility area.

3. After donning disposable shoe covers and gown at the facility entrance, proceed directly to isolation housing and use and complete personnel entry procedures below.

4. Prior to entry, ensure that all portable **equipment and supplies** required for work have been **dedicated to isolation** housing and use.

5. The isolation housing room door must remain locked.

6. Access to isolation housing is limited only to essential personnel with documented didactic tutorial and in-person training presented to the IACUC.

7. Signage on the isolation housing room door must indicate that immunodeficient rodents are housed in isolation and must describe the PPE and procedures required for entrance.

8. Husbandry, care and use of rodents housed in isolation must be **completed first**, before accessing other areas.

9. At the isolation housing room door, all staff must already be wearing a disposable **gown** and **shoe covers** donned at the facility entrance, and then outside the isolation door **don in sequence** (a) **bouffant**, (b) surgical **mask** that covers the

nose, (c) push your thumb through the seam of the sleeve of the gown, (d) put on a pair of **gloves** ensuring cuffs **overlap the gown**, then **enter** the isolation room ensuring shoe covers make **contact** with the **antimicrobial adhesive mat** just inside the door. Before handling anything else, after isolation entry, **don in sequence** (e) **Tyvek sleeves** or apron, and (f) put on a **second pair of gloves** ensuring gloves overlap sleeves and no skin is exposed.

10. **Saturate spray your sleeves and gloves** with Oxivir and wait 5 minutes before handling anything.

III.D. Animal Handling and Use in Isolation

1. Whenever possible, **work in pairs**.

2. Prior to entry, ensure that all reagents and **biologics** have been **characterized** free of infectious agents or are **sterile preparations**, **primary containers** are decontaminated by saturate spraying with Oxivir, and these are placed in nonporous **secondary containers** (e.g., Ziploc bag, conical tube, screw-top containers) that can be surface **decontaminated** by saturate spraying with Oxivir during isolation entry.

3. Supplies and equipment must be **dedicated to isolation** housing and use, and must be decontaminated prior to delivery into isolation.

4. Work in isolation housing and use areas **must precede work in any other animal area**.

5. After donning disposable shoe covers and gown at the facility entrance, proceed directly to isolation housing and use and complete personnel entry procedures above.

6. Each occupied **microisolator** in isolation housing **must remain in its labeled location** on the rack and must be placed in this same rack space **for the duration of study**. Labeling on each cage card should indicate row (i.e., A indicates top row) and column (1 indicates left most) position for each microisolator. A change in microisolator position is possible only when a rack change-out is made (i.e., see item III.E.8, below).

7. Prepare the biosafety cabinet for handling and use of rodents. Ensure the biosafety cabinet is on. **Saturate spray the biosafety cabinet interior with Oxivir** and allow it to sit for 5 minutes.

8. Padded forceps soaking in a beaker or pan of Oxivir solution in the chamber of the biosafety cabinet may be used to assist in the handling of mice.

9. As a **work surface** for instruments, equipment, or supplies, Oxivir soaked paper towels or blue pads may be placed inside the hood.

10. If using portable **equipment** within the biosafety cabinet, ensure these are saturate sprayed with Oxivir, including all surfaces that may come into contact with mice.

11. If inhalational **anesthesia** is planned, saturate spray the induction chambers and rodent nosecone of the quad anesthesia unit and let it sit for 5 minutes.

12. If **imaging** in isolation, disconnect the induction chamber from the anesthesia machine, saturate spray both the interior and exterior of the induction chamber, and enter it into the biosafety cabinet and let it sit for 5 minutes.

13. After ensuring that all supplies and equipment are available and decontaminated, remove a cage with its water bottle in place from the rack and **saturate spray the cage** and water bottle with Oxivir.

14. Place the sprayed cage with bottle in the biosafety cabinet and allow it to sit for 5 minutes. Multiple cages may be sprayed and loaded into the biosafety cabinet, but only one cage may be opened at a time within the biosafety cabinet.

15. **Saturate spray gloves and sleeves** with Oxivir. In addition, a pan of Oxivir solution may be used to decontaminate gloved hands by immersion before opening each microisolator.

16. Ensure that all activities are conducted at least 4 inches within the air curtain of the biosafety cabinet (i.e., greater than the height of a cage card holder).

17. Nothing should touch the interior surfaces of the microisolator, including any surface of the biosafety cabinet.

18. Remove the water bottle and place it on the work surface. Carefully unclamp and remove the microisolator lid and invert it onto the biosafety cabinet work surface and place the wire lid on top of the inner surface of the lid. The wire lid can be used to assist in handling rodents.

19. Complete your work, replace the wire lid and cover on the microisolator, replace the water bottled, clamp the microisolator shut and return it to its same location on the rack.

20. **Between cages, saturate spray gloves and sleeves** with Oxivir. A pan of Oxivir solution in the chamber of the biosafety cabinet may be used to dip gloved hands into and assist in gloved hand decontamination.

21. Whenever mice in **bidirectional** isolation must exit for a **common procedural area**, PPE must be donned in advance in accordance with **entry procedures** described above, the microisolator to be transported is identified and saturate sprayed with Oxivir, and the sprayed microisolator transferred to the common procedural area by **exiting in your PPE**.

22. If **several** microisolators are to be transferred to a common procedural area, a **two person transfer procedure** should be utilized to exit with (a) the interior individual donning PPE in advance in accordance with entry procedures described above, (b)

keeping the door **closed** while the interior individual **identifies and saturate sprays the microisolators** with Oxivir on an interior cart, (c) the door is **opened**, and the sprayed microisolators transferred to the exterior individual and an **exterior cart**, (d) the door is closed, and the exterior cart is used to deliver the microisolators to the common procedure area.

23. If working in **bidirectional** isolation, whenever occupied microisolators are returned to the bidirectional isolation housing room door, a **two person transfer procedure** must be utilized with (a) the interior individual donning PPE in advance in accordance with entry procedures described above, including a **Tyvek suit**, (b) keeping the door **closed** while the exterior individual saturate sprays the microisolators with Oxivir, (c) the door is **opened**, and the sprayed microisolators are transferred to the **interior cart**, (d) the door is closed, and the interior cart and microisolators are **sprayed again** by the interior individual and allowed to sit for 5 minutes inside isolation.

III.E. Husbandry in Isolation

1. Whenever possible, **work in pairs.**

2. Husbandry in isolation housing and use areas **must precede work in any other area**.

3. Sufficient sterile supplies and equipment for change outs must be **delivered in advance** to isolation.

4. After donning disposable shoe covers and gown at the facility entrance, proceed directly to isolation housing and complete personnel entry procedures above.

5. In addition to testing for infectious agents on the exclusion list, rodents in isolation are **monitored for opportunistic bacteria each month** in accordance with SOP 410 Sentinel Rodent Health Surveillance by testing pooled fecal pellets and/or the isolation room environment.

6. Each occupied IVC microisolator in isolation housing must be labeled with its location on the rack and must be placed in this **same space on the rack** for the **duration of study**.

7. After the completion of a study, if a rack position is to be used by a subsequent murine inventory, prior to introducing a new microisolator to the vacated rack position, **decontaminate the air supply and exhaust connections** with an Oxivir wipe.

8. **During IVC rack change-outs**, occupied microisolator inventory can be efficiently regrouped, rearranged, and assigned a new rack position by relabeling cage cards (e.g., covering the old position indicator with a label indicating the new rack position).

9. Husbandry supplies and equipment must be dedicated to isolation housing, must be secondarily enclosed or covered, labeled, sterilized, and delivered into isolation

using the **two person transfer** procedure described above.

10. Individually ventilated caging (IVC) in isolation is **changed weekly**.

11. Any cage excessively soiled is changed as often as necessary to maintain an acceptable level of sanitation.

12. Ensure multiple **padded forceps** are soaking within a beaker or pan of Oxivir solution in the chamber of the biosafety cabinet.

13. Cage changing is noted on the *Room Status Sheet*.

14. IVC microisolators in isolation are **changed as an entire unit, including the bottle** with acidified water, the wire rack top with autoclaved feed, lid and bottom. Feeding and water bottle changing is noted on the *Room Status Sheet*.

15. **Saturate spray gloves and sleeves** with Oxivir

16. Prepare the biosafety cabinet for husbandry. Ensure the biosafety cabinet is on. **Saturate spray every surface of the biosafety cabinet interior** with Oxivir and allow it to sit for 5 minutes.

17. **After uncovering/opening** equipment/supplies needed for change outs (e.g., empty water bottle rack, IVC microisolators with autoclaved feed and bedding, racks of autoclaved water bottles filled with acidified drinking water), **saturate spray with Oxivir** and allow it to sit for 5 minutes.

18. Uncover/open only the supplies/equipment needed for change-outs.

19. While the occupied IVC microisolator to be changed is still on the rack, **remove the water bottle** and place it in an empty water bottle rack that has been previously entered into the isolation housing room.

20. **Saturate spray gloves and sleeves** with Oxivir. When conducting cage change-out procedures, gloves and sleeves are to remain wet with Oxivir.

21. Outer gloves must be changed frequently, in between research cohorts, in between colony lines, in between rack sides, and whenever broken. After changing the outer glove, saturate spray gloves and sleeves with Oxivir.

22. Remove an occupied cage from the rack and saturate spray the cage with Oxivir. Remove a sterile microisolator from the transport rack and **saturate spray the cage** with Oxivir.

23. Place each of the sprayed cages in the biosafety cabinet. **Only one occupied cage may be opened at a time** within the biosafety cabinet.

24. Ensure that all activities are conducted at least 4 inches within the air curtain of the biosafety cabinet (i.e., greater than the height of a cage card holder).

25. Nothing should touch the interior surfaces of the microisolator, including the biosafety cabinet surface. Cage changed-out is one cage at a time and planned so that the microisolator filter top is removed for the least possible amount of time and frequency.

26. Carefully unclamp and remove the microisolator lid of the soiled microisolator and invert it onto the biosafety cabinet work surface and place the wire lid on top of the inner surface of the lid. Similarly, open the clean microisolator.

27. Transfer rodents from soiled to clean microisolator using **forceps**. If handling neonates, pups must be cupped (e.g., in hands or using a scoop previously saturate sprayed with Oxivir) and placed in the new cage.

28. Replace the wire lid and cover on the clean microisolator, clamp the microisolator shut, **spray the closed cage with Oxivir**, and return it to its same location on the rack. Similarly, close the soiled microisolator and place it on the transport rack.

29. **Saturate spray gloves and sleeves with Oxivir between cages**.

30. After cage changing, **saturate spray autoclaved acidified water bottles** required for change-outs with Oxivir. Acidified sterilized water bottles are replaced once a week. Acidified sterile water bottles cannot be refilled. If a water bottle is found to have an insufficient volume, it must be replaced with a freshly sterilized one. Bottle changing is noted on the *Room Status Sheet*.

31. Place a clean autoclaved bottle of acidified water on each clean microisolator.

32. **IVC racks and trolleys** and accessories (e.g., hoses) are changed and sanitized at intervals specified by SOP 400 Rodent Husbandry and memorialized on the IVC Trolley Maintenance Hang Tag, except that IVC racks, hoses, and trolley air handling units are **simultaneously changed at least every 3 months**. The IVC rack changing will be noted on the *Room Status Sheet*.

33. **Soiled caging** is not to be disassembled within the biosafety cabinet or housing room. It is **saturate sprayed with Oxivir** and removed from the room fully intact and taken to the cage wash area for breakdown and sanitation.

34. Work surfaces/hoods are decontaminated after use by saturate spraying with Oxivir and allowed 5 minutes contact.

35. **Depart in your PPE**. If the murine handled inventory is also housed in accordance with SOP 408 entitled "*SOP 408 Biosafety Level-2 (BSL-2) Rodent Husbandry*", remove and dispose of your outer gloves and sleeves prior to departing isolation. If reentry is required, don a **Tyvek suit** and follow the entry procedures described above in item III.C.9-10.

III.F. Animal Handling and Use Outside of Bidirectional Isolation

1. Whenever possible, **work in pairs** when exiting or returning mice to bidirectional isolation after use in a common procedural area.

2. Whenever mice in **bidirectional** isolation must exit for a **common procedural area**, PPE must be donned in advance in accordance with isolation **entry procedures** described above, the microisolator to be transported is identified and saturate sprayed with Oxivir, and the sprayed microisolator transferred to the common procedural area by **exiting in your PPE**.

3. If **several** microisolators are to be transferred to a common procedural area, a **two person transfer procedure** should be utilized to exit with (a) the interior individual donning PPE in advance in accordance with entry procedures described above, (b) keeping the door **closed** while the interior individual **identifies and saturate sprays the microisolators** with Oxivir on an interior cart, (c) the door is **opened**, and the sprayed microisolators transferred to the exterior individual and an **exterior cart**, (d) the door is closed, and the exterior cart is used to deliver the microisolators to the common procedure area.

4. Work in common procedural areas involving mice from bidirectional isolation should **precede work involving other mice** from other housing areas. If multiple cohorts from separate housing rooms are present in the common procedural area, work with cohorts must occur in sequence, with work involving mice from isolation, including those administered uncharacterized human specimens, preceding the other cohort, rather than concurrently. **Only one cage should be opened at a time**.

5. If you have exited bidirectional isolation in your Tyvek suit, dispose of your Tyvek suit after exiting.

6. In the common procedural area, all staff must already be wearing a disposable **gown** and **shoe covers** donned at the facility entrance, and must also don in sequence outside the common procedural room (a) bouffant, (b) surgical mask that covers the nose.

7. Staff working with mice from bidirectional isolation must also then (a) push your thumb through the seam of the sleeve of the gown, (b) put on a pair of **gloves** ensuring cuffs **overlap the gown**, (c) **Tyvek sleeves**, and (d) put on a **second pair of gloves** ensuring gloves overlap sleeves and no skin is exposed.

8. **Saturate spray your sleeves and gloves** with Oxivir and wait 5 minutes before handling anything.

9. **Decontaminate all surfaces that may come in contact with mice**, including the surfaces of the countertop, changing station, biosafety cabinet, anesthesia induction chambers and nosecones, scales, stereotactic surgical instruments, or other portable equipment by **saturate spraying with Oxivir** TB and allow it to sit for 5 minutes.

10. As a **work surface** for instruments, equipment, or supplies, Oxivir soaked paper towels or blue pads may be used.

11. If **imaging**, ensure imaging equipment surfaces that may come into contact with mice are decontaminated with Oxivir in accordance with SOP 1015 *Decontamination of Common Procedural Areas*.

12. After ensuring that all supplies and equipment are available and decontaminated, **saturate spray the cage** and water bottle with Oxivir and allow it to sit for 5 minutes.

13. **Only one cage is to be opened at a time**.

14. **Saturate spray gloves and sleeves** with Oxivir. A pan of Oxivir solution may be used to decontaminate gloved hands by immersion before opening each microisolator.

15. Remove the water bottle and place it on the work surface. Carefully unclamp and remove the microisolator lid and invert it onto the work surface and place the wire lid on top of the inner surface of the lid. The wire lid can be used to assist in handling rodents.

16. Nothing should touch the interior surfaces of the microisolator, even when retrieving a mouse with Oxivir decontaminated gloved fingers.

17. After retrieving a mouse, **before initiating work**, replace the wire lid and cover on the microisolator. After the last mouse from an individual microisolator is used and returned to the cage, also replace the water bottle, and clamp the microisolator shut.

18. **Between cages, saturate spray gloves and sleeves** with Oxivir.

19. Whenever occupied microisolators are **returned** to bidirectional isolation housing after working in a common procedural area, a **two person transfer procedure** must be utilized with (a) the interior individual donning PPE in advance in accordance with entry procedures described above, including a **Tyvek suit**, (b) keeping the door **closed** while the exterior individual saturate sprays the microisolators with Oxivir, (c) the door is **opened**, and the sprayed microisolators are transferred to the **interior cart**, (d) the door is closed, and the interior cart and microisolators are **sprayed again** by the interior individual and allowed to sit for 5 minutes inside isolation.

Approved: **Date:**