

STANDARD OPERATING PROCEDURES
DIVISION OF COMPARATIVE MEDICINE
UNIVERSITY OF SOUTH FLORIDA

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TITLE: **Animal Biosafety Level-2 (ABSL-2) Rodent Husbandry**
SCOPE: All Authorized Personnel
RESPONSIBILITY: Facility Manager and Technical Staff
PURPOSE: To Outline the Proper Procedures for Safe Husbandry Practices for Rodents Housed at ABSL-2.

I. PURPOSE

1. To outline the proper procedures for safely conducting husbandry of rodents housed under animal biosafety level 2 (ABSL-2) conditions.
2. Reduce the risk of exposure of research and animal care staff to biohazardous agents within the animal facility.

II. RESPONSIBILITY

1. The Facility Manager is responsible for ensuring:
 - a. That staff contributing to husbandry are adequately trained to perform the husbandry practices described.
 - b. Implementation of the procedures described.
 - c. Dedicated ABSL-2 rooms for infectious agents, and animal housing rooms for rDNA and PDX biocontainment at the cage level are clearly labeled with the biohazardous agent present and specific safety practices implemented.
 - d. Appropriate personal protective equipment (PPE) is available.
 - e. Biohazard Safety Data Sheets (SDSs) are accessible, when available.
 - f. Safety practices have been communicated to the relevant personnel.
2. It is the responsibility of the animal care staff to:
 - a. Read, understand, and follow the procedures described.
 - b. Don appropriate PPE and review room signage and SDS prior to implementing the procedures described.

III. PROCEDURES

1. Containment practices are in accordance with Institutional Biosafety Committee (IBC) protocol-specific requirements for the agent in use. Biohazard signage used on the animal housing room door and biohazard flags used on the cage must be posted and removed in a timely manner, reflective of the actual use of biohazardous agents in the room.
2. Typically, containment of replication deficient viral **recombinant DNA** constructs or primary human patient-derived xenografts (**PDX**) administered to mice or rats is at the **cage level** in a conventional animal housing room with (A) signs **SC #247** and **SC #251** posted on the outside of the door, (B) all primary enclosures administered

such agents identified with an **orange biohazard cage card flag** indicating agent administered, exposure date, release date, PI, and emergency researcher contact information, and (C) grouped together on the rack. A class II **biosafety cabinet** (BSC) is utilized for cage change-outs and procedures, or if a changing station is utilized, additional PPE is required. Eye shielding and mask are required when working outside a BSC.

3. In contrast, typical containment of replication competent **infectious agents** administered to mice or rats is accomplished in a **separate, dedicated ABSL-2 containment room** with (A) **sign SC #252** on the door **indicating the infectious agent(s) used within** (B) primary enclosures are identified with an **orange biohazard cage card flag** and (C) grouped together on the rack. All cage change-outs and procedures involving infectious agents are performed in a **BSC in the ABSL-2 animal housing room**.
4. Before entering the room, **assemble all supplies** necessary to complete task.
5. Access to rooms housing ABSL-2 rodents is **limited to essential personnel**. **Doors are kept closed** when experimental animals are present, and when appropriate, **locked** when the room is unattended.
6. Husbandry and care of ABSL-2 rodents are **completed after other colonies**.
7. **Before entering the room review ABSL-2 signage posted on the door** for PPE requirements and specific practices implemented for the protocol/agent.
8. When ABSL-2 rodents are not housed in a separate room (e.g., primary enclosures of mice administered recombinant DNA or PDX), ABSL-2 cages shall be grouped together and individually identified with an **orange biohazard cage card flag** indicating the agent in accordance with SOP 006.
9. Don a disposable gown, gloves, sleeves and shoe covers prior to entering the room. Additional PPE as required by signage must also be donned, which may include but is not limited to eye protection, face shields, and respirators.
10. **Work surfaces are cleaned and decontaminated** with Oxivir Tb before and after use.
11. **Cage changing of infectious agent administered rodents must be performed within a BSC**.
12. **Cage changing of rDNA or PDX administered rodents should be performed within a BSC** (see **SOP 1127**) during the IBC interval of agent containment (e.g., **first change** after 3 or 7 days for replication deficient adeno- or lenti-viral **recombinant DNA** constructs, respectively) when present or whenever there is a high potential for creating aerosols. Eye shielding and mask are required when working outside a BSC (e.g., changing station). As a rule of thumb:
 - a. Intervals of cage change-outs will be determined based on the associated health risk of the agent and animal housing density.
 - b. Cage changing is performed twice a week for all static microisolator shoebox caging when housing rodents at standard densities.

- c. Cage changing for single housed mice in static microisolators may be performed at intervals longer than twice weekly, but should at least be performed once a week.
 - d. Individually ventilated caging (IVC) is routinely performed at intervals of once every two weeks for most rodents, every week for immune deficient mice, and as needed for rodents with special needs (i.e., diabetic that exhibit increased urine output).
 - e. Due to scant urine production, IVC caging is routinely changed every three weeks for gerbils.
 - f. Only a single occupied microisolator should be placed in the BSC at a time.
 - g. Rodent **caging is changed-out one cage at a time** and husbandry practices are planned so that the microisolator filter top is removed from the shoe-box cage for the least possible amount of time and frequency.
 - h. Any cage excessively soiled will be changed as often as necessary to maintain an acceptable level of sanitation.
 - i. Cage changing will be noted on the **Room Status Log**.
13. Occupied microisolators to be returned to the rack, and gloved hands, are sprayed with Oxivir Tb and allowed the appropriate contact time for disinfection (i.e., 1 minute for Oxivir Tb) **prior to removing from the BSC**.
14. All procedures are carefully performed to **minimize the creation of aerosols**.
15. **Water bottles, sipper tubes and stoppers are sent to the cagewash after autoclaving for weekly sanitation.** Water bottles are removed from occupied microisolators within the BSC and are racked in a clean microisolator with lid or in a water bottle rack with Tyvek cover in preparation for autoclaving. Water bottles will be checked daily and replaced as needed to ensure adequate supply is available. **Changing of water bottles is recorded on the Room Status Sheet.**
16. Soiled microisolator **caging is autoclaved as a unit prior to transferring to cagewash for sanitation.** To prepare soiled caging for autoclaving:
- a. **Remove microisolator top**, empty feed from wire rack. Replace wire and lid and lock cage.
 - b. **Ensure water bottle** is capped and place in the wire basket. Once the basket is full, cover with the Tyvek cover to be autoclaved.
 - c. **Place a Verify® Integrator strip in a representative cage for each batch of cages to be autoclaved in a decontamination cycle** so that it can be readily observed and replace the microisolator top. Do not clamp IVC microisolator tops.
 - d. When an autoclave is not located within the BSL-2 room:
 1. Place caging unit in an autoclavable bag. Note: the bag must NOT be a bag labelled as *biomedical* or *biohazardous waste*.
 2. **Seal bag** (e.g., tape or twist tie), and place autoclave indicator tape on outside of bag. Note in which bagged cage the Verify Integrator strip has been placed by noting on the autoclave tape.
 3. **Spray outside of bag** with bleach solution (1:10 dilution), or Oxivir Tb and allow for appropriate contact time for disinfection (i.e., 5 minutes for Oxivir Tb and 2 minutes for bleach) prior to removing from the room.
 4. **Transport bag directly to autoclave.**

5. Autoclave sterilization is performed in accordance with **SOP #1006** and monitored in accordance with **SOP #1002**.
 - d. When autoclaved caging is ready for exit from the ABSL-3 satellite facility and to be removed from the pass-through autoclave in IDRB room 428.
 1. The biological indicator is removed from the simulated “test” load and incubated in accordance with **SOP #1007**.
 2. The biohazardous load is securely stored in the dedicated locked freezer in IDRB room 428 until evidence that the biological indicator from the simulated load indicates that the biohazardous load has been successfully decontaminated.
 3. Results of monitoring are recorded on the **Autoclave Sterilization Record**.
 4. Autoclaved caging/equipment can only be removed from the locked freezer in IDRB room 428 and taken to IDRB cage wash room 406 for sanitation after efficacy of decontamination has been confirmed.
17. **Work surfaces are decontaminated** after each use with bleach solution (1:10 dilution), followed by 70% alcohol rinse. Alternatively, an accelerated hydrogen peroxide (i.e., Oxivir Tb) can be used.
18. **Protective clothing is removed and discarded in a red biohazardous container within the room/suite.**
19. **Hands are washed/sanitized prior to leaving the room/suite.**
20. **When biohazardous waste containers are to be removed/emptied containers/bags are sprayed** with bleach solution (1:10 dilution), or Oxivir Tb and allow for appropriate contact time for disinfection (i.e., 5 minutes for Oxivir Tb and 2 minutes for bleach) prior to removing from the room.
21. Room Duties
- a. Daily room duties: Upon completion of cage changing, feed and watering, and daily health surveillance, the following tasks are to be performed: (Room duties described below are minimal requirements, additional duties and frequencies are at the Facility Manager’s discretion).
 1. Complete **Room Status Sheet** by recording:
 - a. Minimum and maximum room or IVC rack temperature and humidity (measurements out of the acceptable range are described under the **Health and Environmental Concern** column of the **Room Status sheet** and reported to the Facility Manager via the **Health and Environmental Concern Form**).
 - b. Feed and water are available.
 - c. Ventilation trolleys are functional (for IVC caging)
 - d. Caging and equipment changes performed.
 - e. Housekeeping duties performed.
 - f. Significant health or environmental concerns.
 - g. Time of observation and the initials of technician.
 2. Clean/disinfect floor with Decon-Spore 200 Plus using a mop handle with a disposable cleaning pad/head.
 3. Assure no investigator’s supplies or trash is left in the room.
 4. Check and replenish supplies (e.g., soap, paper towels) as needed.

5. Wipe down counter/sink areas with Oxivir Tb
6. Assure any new arrivals are recorded on appropriate ***Per Diem Sheets***.
7. Record any additions or subtractions made by room technician or research staff as observed (e.g., weanlings, breeding pairs set up/removed, euthanasia, etc.)
- b. **Weekly** room duties include:
 1. Clean and disinfect changing station, if present, and clean/replace filters in accordance with **SOP #1129** entitled ***NuAire Small Animal Cage Changing Station***.
 2. Clean and disinfect biosafety cabinets, if present, in accordance with **SOP #1127** entitled ***Biological Safety Cabinets***.
 3. Wipe down door and door-frame with Oxivir Tb.
 4. Physically count animals within the room.
- c. **Semi-monthly** room duties include:
 1. Sanitize all room cleaning equipment within the room with, Oxivir Tb (e.g. wipe down mop handle, dust pan, etc.).
- d. **Monthly** room duties include:
 1. Clean HVAC vents.
 2. Confirm diurnal light timers are accurately controlling animal room lights **SOP 1145 HOB0 Data Logger**, and record.
- e. Room surfaces and equipment are disinfected at the end of a study.

22. Animal Relocation Procedures

- a. Whenever rodents are to be relocated for use of common procedural areas outside of the ABSL-2 housing room (e.g., small animal imaging core, multiphoton microscopy core):
 1. Relocation is scheduled by Comparative Medicine staff and accomplished when no other animals or activities are present in the immediate area.
 2. Relocation is accomplished in clean primary enclosures, the exterior surface of which is decontaminated with Oxivir Tb prior to being removed from the housing room by Comparative Medicine staff.
 3. Surfaces and common equipment are decontaminated before and after use in accordance with **SOP 1015 Decontamination of Common Procedural Areas**.
 4. After return to ABSL-2 housing, primary enclosures used in transit are decontaminated by autoclaving.

23. Complete cleaning and sanitization of the IVC racks at the IDR facility requires that it be relocated to a facility with a rack-washer. Prior to removing the IVC rack from the BSL-2 suite the rack must be prepared for transport by following the decontamination and containment procedures below:
 - a. While wearing appropriate PPE, remove all caging from rack being removed and place on the IVC replacement rack.
 - b. Animals/cages will be moved to the new rack so that they retain the exact same cage position as they held on the original rack.
 - c. Spray rack with Oxivir Tb thoroughly and allow for 5-minute contact time.
 - d. Wrap rack with cellophane/plastic wrap thoroughly covering all surfaces so that rack is completely contained with only the wheels exposed.
 - e. Spray wheels with Oxivir Tb as the rack is being removed from the room.

- f. Remove PPE prior to exiting the room.
- g. Roll rack to freight elevator and load onto vehicle for transport to rack-washer.
- h. Upon arrival to the facility for sanitization, the rack is rolled directly into the dirty-side of cage wash.
- i. Personnel don appropriate PPE.
- j. Cellophane wrap is removed and placed in red bag for disposal as biohazardous waste.
- k. Supply and exhaust nozzles are removed and placed in Oxivir Tb solution.
- l. Supply and exhaust plenums are removed, and plenum caps/sleeves are placed in Oxivir Tb solution.
- m. Exhaust plenum is disconnected and flushed with a hose to remove debris.
- n. Rack is loaded into rack-washer and run. The rack is inspected and may be cycled through the rack-washer a second time if necessary.
- o. Clean rack is reassembled using the sanitized components.

24. Animals administered uncharacterized primary human tumor resections, tissue explants, blood, or other primary human or patient-derived xenografts (PDX) are housed in ABSL-2 containment. Xenografts that were primary human-derived may be characterized by polymerase chain reaction (PCR), enzyme linked immunosorbent assay (ELISA), or other appropriate methodology care of Comparative Medicine prior to implantation or during passage of xenografts from F1 to F2 recipient mice. If such grafts are shown to be free of human agents of concern, the characterized recipient mice are eligible for housing at ABSL-1, at the determination of the veterinarian. Human agents of concern that primary human-derived specimens must be tested for and found to be absent of in order to eligibilize recipient mice for housing at ABSL-1 include Mycoplasma spp., Human immunodeficiency virus 1 (HIV1), Human immunodeficiency virus 2 (HIV2), Human T-lymphotropic virus 1 (HTLV1), Human T-lymphotropic virus 2 (HTLV2), Hepatitis virus A, Hepatitis virus B, Hepatitis virus C, Hantaviruses (Hantaan, Seoul, Sin Nombre viruses), Herpes simplex 1, Herpes simplex 2, Human cytomegalovirus, Human herpes virus 6, Human herpes virus 8, Human adenovirus, Human papillomavirus 16 & 18 (HPV), Lymphocytic choriomeningitis virus, Varicella virus, Epstein-Barr virus, and Treponema pallidum. Test results or written assurances provided by other diagnostic laboratories or sources of primary human specimens (e.g., blood bank) may be considered by CM veterinarians in conjunction with the biosafety office to determine whether an acceptable assurance of the absence of hazard is provided to make recipient animals of the specimen eligible for housing at ABSL-1.

Approved:

Date: