STANDARD OPERATING PROCEDURES

DIVISION OF COMPARATIVE MEDICINE
UNIVERSITY OF SOUTH FLORIDA

TITLE:	**Animal Biosafety Level-3 (ABSL-3) Husbandry at the Interdisciplinary Research Building (IDRB)**
SCOPE:	All Authorized Personnel
RESPONSIBILITY:	Facility Manager and Technical Staff
PURPOSE:	To Outline the Proper Procedures for Safe Husbandry Practices for Animals Housed at ABSL-3.

I. PURPOSE

1. To outline the proper procedures for safely conducting husbandry of animals housed under animal biosafety level 3 (ABSL-3) containment.

2. Reduce the risk of exposure of research and animal care staff certified to work with infectious agents within the ABSL-3 facility. Agents classified as requiring BSL-3 containment may have serious or potentially lethal consequences as a result of exposure.

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 and familiar with ABSL-3 practices including occupational health and safety practices and/or recommendations.
    b. Implementation of the procedures described.
    c. The exit door from the gown-in Room 416 and animal housing Room 421 are clearly labeled with the biohazardous agent(s) approved for use in Room 421, and the specific safety practices implemented.
    d. Appropriate personal protective equipment (PPE) is available.
    e. Biohazard MSDSs are accessible when available.
    f. Safety practices have been communicated to the relevant personnel.

2. It is the responsibility of the ABSL-3 certified animal care staff to:
    a. Complete enrollment in the occupational health and safety program prior to working within the ABSL-3 facility.
    b. Read, understand, and follow the procedures described.
    c. Don appropriate PPE and review room signage and MSDSs prior to implementing the procedures described.
    d. Have specific understanding of the safe handling of infected animals, sharps, and waste material in the ABSL-3 setting.

III. PROCEDURES

1. Access to ABSL-3 facilities is limited to appropriately trained, certified, and authorized personnel.

2. Donning of appropriate BSL-3 PPE is conducted in Anteroom Room 416 in the following sequence:
    a. When wearing the half Mask Respirator:
        1. Tyvek suits
        2. Inner gloves
        3. Apron
        4. Outer gloves
        5. Respirator
        6. Goggles or face shield
        7. Head cover
    b. When wearing the PAPR:
        1. Battery belt and battery
        2. Tyvek suits
        3. Inner gloves
        4. PAPR
        5. Apron
        6. Outer gloves

3. **Before exiting gown-in 416 review BSL-3 signage posted on the door** for PPE requirements of Room 421 and specific practices implemented for the protocol(s)/agent(s) present.

4. After exiting gown-in room 416 and before entering Room 421, **assemble all supplies** necessary to complete task. Husbandry supplies are maintained in Room 417.

5. **Work surfaces within the Class II biosafety cabinet (BSC) are decontaminated** with a 10% bleach solution, followed by 70% ethanol rinse, prior to use.

6. Rodents and chicks of appropriate size may be housed in individually ventilated caging (IVC). Approved amphibian, reptile, and avian species may utilize static microisolator caging as a primary enclosure when maintained in an environmentally controlled chamber capable of temperature and diurnal regulation.

7. **Only essential items/equipment should be placed in the BSC for the intended procedure.** Clean items should be segregated from ones that may become contaminated within the BSC and **flow of work should be from clean to dirty**.

8. **All husbandry and technical procedures are conducted in a Class II BSC in accordance with SOP 1127.**
    a. Intervals of cage change-outs will be determined based on the species involved, associated health risk of the agent, and animal housing density.
    b. Cage changing for animals housed in IVC microisolators may be performed at intervals longer than once a week, but should at least be performed every 2 weeks.
    c. **Only a single occupied cage should be placed in the BSC at a time**.

d. **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.
e. Any cage excessively soiled will be changed as often as necessary to maintain an acceptable level of sanitation.
f. Soiled **caging is changed and autoclaved prior to sanitation.**
g. Cage changing will be noted on the ***Room Status Sheet***.
h. IVC racks or environmental chambers are sanitized at intervals determined by the Facility Manager and the frequency is determined based on the species involved, associated health risk of the agent, and animal housing density.

9. Occupied microisolators to be returned to the IVC rack or environmental chamber, and gloved hands, are sprayed with 10% bleach solution and allowed the appropriate contact time for disinfection(i.e., 2 minutes) followed by 70% ethanol rinse **prior to removing from the BSC**.

10. All procedures are carefully performed to **minimize the creation of aerosols**.

11. **Water bottles, sipper tubes and stoppers are changed-out weekly in the BSC and autoclaved out of the ABSL-3 facility, and when decontamination is assured, are sent to the PSY cagewash for sanitation.** Water bottles will be checked daily and replaced as needed to ensure adequate supply is available. Changing of the bottles is **recorded on the *Room Status Sheet*.** Occupied microisolators, and gloved hands, are sprayed with 10% bleach solution, and allowed the appropriate contact time for disinfection, followed by 70% ethanol rinse **prior to removing from the BSC**.

12. When preparing to change-out microisolators, prior to handling soiled caging create a simulated “test” load by **first** placing a biological indicator deep within a clean “test” pack that consists of the identical components (i.e., same number of cages/equipment, bedding, and water content) as the anticipated soiled pack to be autoclaved. The test pack and biohazardous pack should be **clearly identified** on the outside of the pack.

13. Soiled microisolator **caging is changed and autoclaved prior to transferring to PSY cage-wash for sanitation**. All procedures to prepare soiled caging for autoclaving are performed under a Class II BSC:
    a. When performing cage-changing at the termination of a study (i.e., animal occupying cage is euthanatized or found dead), caging is changed-out as a unit**:**
        1**. Remove microisolator top**, empty feed from wire rack and the contents of the water bottle onto the soiled cage bedding and place uncapped water bottle in microisolator bottom.
        2. **Place a Verify® Integrator strip in the cage** so that it can be readily observed.
        3. **Replace the microisolator top loosely on cage** and place unit(s) in an autoclavable biohazard bag.
    b. When performing cage-changing during a study (i.e., animal to be returned to IVC rack after cage changing), only cage bottoms are changed-out:

1. Microisolator top and wire rack are not changed-out but are placed on a clean bedded microisolator bottom along with clean water bottle and fresh feed as needed.
2. Empty the contents of the used water bottle onto the soiled cage bedding and place uncapped water bottle in microisolator bottom.
3. **Place a Verify® Integrator strip in the cage** so that it can be readily observed.
4. Place microisolator bottom in an autoclavable biohazard bag.

c. **Double bag all items to be autoclaved, then seal** (e.g., tape or twist tie) the outer bag, and place autoclave indicator tape on outside of bag.
d. **Spray outside of bag and gloved hands** with 10**%** bleach solution and allow for appropriate contact time for disinfection prior to removing from Class II BSC.
e. **Transport bag directly to the pass-through autoclave** in Room 427**.**
f. **Autoclaving of biohazardous waste is performed in Room 427.**

14. **Work surfaces of the Class II BSC are decontaminated** after each use with 10% bleach solution, followed by 70% ethanol rinse.

15. Exiting ABSL-3 Room 421
    a. When removing waste from the room:
        1. Remove outer gloves and **don new gloves prior to exiting Room 421**. Outer gloves are disposed of as biohazardous waste.
        2. Exit to the autoclaves located in dirty corridor Room 427.
        3. Load autoclave in accordance with autoclave SOP.
        4. Remove and dispose of outer gloves and plastic apron in biohazard waste receptacle near autoclave in Room 427.
        5. Exit back through dirty corridor to room 424 and continue through Room 424 to clean corridor Room 418.
        6. Just prior to airlock Room 416 remove and dispose of tyvek suit.
        7. In airlock Room 416 **remove remaining PPE in the following order**:
            a. Mask or PAPR. Clean/disinfect half-face mask or PAPR with Vesphene® llse.
            b. inner gloves and PAPR if used
        8. Exit to anteroom Room 415
            a. Place battery on charger if PAPR was used.
            b. **Wash hands with soap and water**.
        9. **Sign out** Entry Exit Log
    b. When exiting without waste for autoclaving:
        1. **Remove outer gloves and apron prior to exiting Room 421**. Outer gloves and apron are disposed of as biohazardous waste.
        2. Exit through dirty corridor Room 425 and Room 424 to clean corridor Room 418.
        3. Just prior to airlock Room 416 remove and dispose of tyvek suit.
        4. In airlock Room 416 **remove remaining PPE in the following order**:
            a. Mask or PAPR. Clean/disinfect half-face mask or PAPR with Vesphene® llse.
            b. inner gloves and PAPR if used
        5. Exit to anteroom Room 415
            a. Place battery on charger if PAPR was used.
            b. **Wash hands with soap and water**.

6. **Sign out** Entry Exit Log

16. Autoclaved caging is removed from the pass-through autoclave in Room 413.
    a. The biological indicator is removed from the simulated "test" load and incubated in accordance with the instructions in ***SOP #1007.***
    b. The biohazardous load is securely stored in a dedicated locked freezer in Room 413 until evidence that the load has been successfully autoclaved as indicated by the biological indicator for the simulated load.
    c. Results of monitoring are recorded on the ***Autoclave Sterilization Record***.
    d. Autoclaved caging/equipment can only be removed from the facility and taken to PSY cagewash for sanitation after efficacy of sterilization has been confirmed and recorded. **The mechanical cage-washer has a final rinse temperature of at least 180^0F.**
    e. A dedicated container is used to relocate autoclaved caging/equipment out of the IDRB facility.

17. **When biohazardous waste containers are to be removed/emptied, containers/bags are sprayed** with 10% bleach solution and allowed appropriate contact time for disinfection prior to removing from Room 421.

18. **All biohazardous waste is autoclaved prior to disposal in the BSL-3 waste receptacle located in Room 413.**

19. IVC accessories are changed/sanitized at intervals determined based on animal housing density and associated health risks of agents with consideration of the following intervals when run continuously and occupied at maximum capacity. IVC maintenance is memorialized on the ***Ventilated Cage System Maintenance Log***:
    a. Cage pre-filters- check at each cage change-out and change when visibly soiled or after autoclaving.
    b. Cage HEPA filters- change after autoclaving
    c. Air handling unit (AHU) pre-filters- change every 3 months
    d. Trolley certification- 12 months
    e. AHU Supply and exhaust HEPA filters- as needed (based on vendors recommendation during annual certification).
    f. Trolley decontamination- as needed (based on vendors recommendation during annual certification).
20. Room Duties
    a. 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 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. Caging and equipment changes performed.
            d. Housekeeping duties performed.
            e. Significant health or environmental concerns.

   f. Time of observation and the initials of technician.
2. Check and replenish supplies (e.g., soap, paper towels) as needed.
3. Wipe down counter/sink areas with 10% bleach followed by 70% ethanol as needed.
4. Assure any new arrivals are recorded on appropriate ***Per Diem Sheets.***
   a. Record any additions or subtractions made by room technician or research staff as observed (e.g., weanlings, breeding pairs set up/removed, euthanasia, etc.)
5. Monthly confirm diurnal light timers are accurately controlling animal room lights, and record.
6. Room surfaces and equipment are disinfected at the end of a study and/or as needed.

Approved: **Date:**