**STANDARD OPERATING PROCEDURES**

# 1. Performance Standard: Practices and procedures used when working with animals at the ABSL2 level are in accordance with those described in this SOP and the CDC-NIH Biosafety in Microbiological and Biomedical Laboratories.

# Standard Practices

* All personnel working at the ABSL2 hazard level are required to attend the Department of Laboratory Animal Medicine (DLAM) BSL2 training program. The DLAM staff attends an ABSL2 training. The DLAM and Bio Safety Officer will provide additional training as needed. The CDC/NIH Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, is used as the basis for education and training.
* Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use **are not permitted in the animal facilities**.
* Access to the animal facility is limited or restricted to personnel who have been advised of the potential hazard and who need to enter the room for program or service purposes when work is in progress. Access is at the discretion of the Director of Laboratory Animal Medicine or Facility Manager. The PI is responsible for advising his/her personnel on the potential risks due to the hazards involved in the project. The DLAM is responsible for advising its personnel on the potential risks due to the hazards involved in the project. Persons who may be at increased risk of acquiring infection, or for whom infection might be unusually hazardous, are not allowed in the animal room.
* All personnel must receive appropriate immunizations or tests for the agent handled or potentially present (e.g., hepatitis B vaccine or TB skin testing). The Occupational Health Physician/CDC/Health Department will be consulted as appropriate, depending on the agent under study and the potential risk to personnel.
* When appropriate, considering the agent handled baseline serum samples from animal care and other at‑risk personnel may be collected and stored. Additional serum samples may be collected periodically depending on the agent handled or the function of the facility.
* A study requiring ABSL2 practices is performed in an animal room dedicated for this purpose after discussion between the Principal Investigator (PI) and the DLAM Director or Facility Manager.
* Research personnel are required to
* be listed on an IACUC approved protocol,
* have completed a Medical Health Questionnaire Form,
* be enrolled at the appropriate level in the UNTHSC Occupational Health Program,
* have attended the DLAM Animal/Policy Training and tour for access into the animal facility and
* have received individual training from the DLAM staff for entering and working in an ABSL2 room.
* An IACUC approved protocol describing the procedures and a consult with the BioSafety Officer for Safety concerns for the study are required prior to ordering animals on any protocol involving hazards.
* The DLAM training must be completed and the lab must be in compliance prior to the start of the ABSL2 work.
* The PI/research staff are responsible for putting a biohazard sticker with the number corresponding to the agent on the cage card.
* The DLAM Facility Manager is responsible for
* posting a hazard warning sign incorporating the universal biohazard symbol on the access door to the animal room when an infectious agent is in use in the animal room. The hazard warning sign identifies the infectious agent(s) in use; lists the name and telephone number of the responsible animal facility personnel, the PI, and other responsible person(s); and indicates the special requirement(s) for entering the animal room.
* Use caution when handling contaminated sharp items (e.g. needles and syringes, slides, pipettes, capillary tubes, and scalpels). Substitute plastic ware for glassware whenever possible.
* Use only needle‑locking syringes or disposable units where the needle is integral to the syringe.
* Syringes that re-sheathe the needle, needleless systems, and other safe devices should be used when appropriate. Training for use of these systems is provided by the DLAM veterinary staff as necessary.
* Do not be bend, shear, break, recap, remove or otherwise manipulate by hand needles from disposable syringes. Carefully place them in the sharps container provided by the DLAM staff.
* Place non‑disposable sharps into a hard‑walled container for transport to a processing area for decontamination. (Glass bottle, polycarbonate/polysulfone, neoprene)
* Do not handle broken glassware directly by hand. Remove it by mechanical means such as a brush and dustpan, tongs, or forceps.
* Containers of contaminated needles, sharp equipment, and broken glass should be decontaminated before disposal, according to the DLAM decontamination procedures.
* Place cultures, tissues, or body fluid specimens in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping.
* Contaminated equipment must be decontaminated according to local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state or federal regulations before removal from the facility.
* Decontaminate cages and accessories before they are brought to the Dirty room. Autoclaving is the preferred method of decontamination. If autoclaving is used, perform biological monitoring on a weekly basis to verify cycle parameters. If autoclaving is not practical or possible, thoroughly decontaminate cages and accessories with Sporklenz (for rodents)
* Sporklenz is pre-mixed and ready to use. A minimum of 10-minutes contact time is required.
* Equipment and work surfaces should also be decontaminated using paper towels saturated with Sporklenz, after work with infectious materials is finished and especially after overt spills, splashes, or other contamination by infectious materials. Wear gloves when using Sporklenz. Dispose of the gloves and paper towels in the biohazard trash.
* Perform all procedures carefully, to minimize the creation of aerosols. Aerosol-producing situations include routine husbandry procedures and inoculation of animals with infectious organisms. Regarding husbandry procedures, the following precautions should be taken to minimize aerosol production:
* Floors are cleaned by application of bleach solution to the floor (1/2 cup of bleach in 1 gallon of water),. Change mop head every month.
* Soiled Tubs and Bedding from animals in the ABSL2 are autoclaved prior to disposal in the dirty room. Any soiled caging is to be placed in the red bio bubble in 024. Leave a microisolator top on top of the open tub in a stack to prevent contamination. Spray the stack with sporklenz and close the red bio bubble. Do not remove soiled caging from the red bio bubble. DLAM personnel will remove it and place it in the autoclave before removing it from the BSL suite.
* Aerosol-producing activities, including necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, intranasal inoculation of animals, and manipulations of high concentrations or large volumes of infectious materials, must be done in a biological safety cabinet unless special precautions have been approved in advance.
* Double-bag all wastes that can be effectively autoclaved Spray the outside bag with Sporklenz.
* Place bags that don’t require refrigeration/freezing in an infectious trash can.
* Places bagged carcasses in the cooler/freezer labeled as a storage site for biohazard samples.
* Personnel must remove their gloves and thoroughly wash their hands after handling cultures and animals, and before leaving the BSL suite and DLAM Facility. Hands must be washed with an antimicrobial soap for at least 30 seconds, in the nearest sink, prior to leaving the DLAM. The DLAM provides a hand sanitizer when a sink is not available in the animal facility.
* An insect and rodent control program is in effect. The routine DLAM pest management program applies to all areas where ABSL 2 studies may be performed.

# Special Practices

* The study will not begin until training specific to the study is completed and documented by the DLAM. Personnel receive annual updates, or additional training as necessary for procedural or policy changes. The Compliance and Quality Assurance Coordinator and maintains records of all training, as per CFR 1910.1030.
* Animals not involved in the biohazardous study are not permitted in the animal room.
* All equipment leaving the room is appropriately decontaminated as described in section 2 of this SOP, above.
* Animals are housed in primary biosafety containment equipment (ventilated negative air flow racks) appropriate for the species.
* Spills and accidents that result in overt exposures to infectious materials must be immediately reported to the DLAM, Safety, and to the PI. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

1. **Animal Facility**

* The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. Access to the facility is limited by a key-card access security system.
* The DLAM facilities are designed and constructed to facilitate cleaning and housekeeping.
* A handwashing sink is available within the animal facility where infected animals are housed.
* Exhaust air is discharged to the outside without being re-circulated to other rooms. The direction of airflow in the animal room is inward.
* An autoclave that can be used for decontaminating infectious waste is available within BSL suite.

# 5. Standard Personal Protective Equipment (PPE)

* All personnel entering animal rooms housing ABSL2 agents must wear the following PPE:
* disposable gown,
* Latex or nitrile gloves (double gloves are worn when there is a potential of scratch hazard),
* shoe covers
* bouffant cap and a
* face mask (DLAM Staff are required to wear an N95 mask).
* NOTE: PPE is put on upon entering the BSL suite, and removed immediately prior to exiting the suite. PPE is disposed of as infectious waste.

**7. Transfering animals from housing room to procedure rooms (15A, B, E)**

To transfer the animals from the housing room to procedure rooms first wipe down the top of the transport cart with sporklenz. Remove the necessary tubs intact with lid from the rack. Place on transport cart. Spray down wheels with sporklenz before exiting the housing area. All infectious animal tubs must be opened and manipulated in the biosafety cabinet.(See SOP #8 for proper use of biosafetycabinet) When finished place the tub back on the cart. Spray wheels down before exiting procedure room. Remove animals from cart and place back on rack in proper place. Ensure that cage is properly aligned and secure before leaving the room. Spray wheels down before exiting ABSL2 Suite.

**CARE AND USE OF CAGE CHANGING HOODS**

**I. PURPOSE**

A laminar flow Class II, Type A/B3 biological safety cabinet is valuable supplement to good sterile technique, providing a sterile work environment as well as protecting personnel from exposure to dust and dander. This standard operating procedure describes how to utilize the cabinet correctly to provide an adequate barrier. There are two brands of portable biological safety cabinets used for cage changing in the Division: Allentown’s Biogard Class II Type A and Nuaire’s Labgard Class II Type A/B3.

**II. RESPONSIBILITY**

The PI and Facility Manager are responsible for training staff members to use change hoods correctly and properly change cages within the hood.

Staff members should keep their hoods clean and report any problems with the hood to the supervisor.

The Room Attendant should inspect and clean each biological safetycabinet monthly.

The Facility Manager must arrange annual certification of each unit’s HEPA filters to assure continued sterile work environments.

**III. PROCEDURE**

1. Make sure that the blower is on, if not turn on. To assure there is airflow, listen for the sound of the blower motor. Most units have a magnahelic gauge, which measures positive pressure. This should register above zero and should be consistent with the recommended level listed on the AirScan Services Certification sticker affixed to the unit.
2. Turn on the fluorescent light. Some older units have an ultraviolet light option. Make sure the ultraviolet light is turned off.. Extended viewing of an ultraviolet light can cause eye damage.
3. Each biosafety cabinet provides a curtain of vertical, sterile air flowing down the sash opening. Consider everything inside the cabinet workspace behind this vertical curtain as sterile. When properly gowned and gloved wipe down the interior area of the cabinet with a surface disinfectant such as Clidox, Spor-klenz, or fresh 10% bleach solution.
4. Place all materials to be used inside the cabinet. In most cases cabinets are used for changing sterile cages, so be sure to disinfect the exterior of these materials, wiping down with a cloth or wipe wet with disinfectant. Everything required (and nothing more) should be placed in the cabinet before beginning work so that nothing will pass in or out through the air barrier until the procedure is completed. Within the work area, implements should be arranged in logical order so that clean and dirty materials are segregated, preferably on opposite sides. Blocking the front and rear perforated grilles must be avoided. If wipes are used, be sure to keep them away from the grilles.
5. If needed, the glass view screen can be opened to place materials in the workplace. After materials are in place, inside the cabinet, close the view screen. Never operate the cabinet with the view screen in any other position. The twelve-inch opening is essential for proper operation. The alarm will sound if the view screen is improperly used.
6. Once the view screen is in proper position, allow the cabinet to operate for three minutes to purify the workplace air.
7. Staff members should wear disposable gowns with tight fitting cuffs and gloves. This minimizes the shedding of skin flora into the work area and protects hands and arms from contamination.
8. Perform all work on the work surface keeping arms inside the air barrier. Work with a limited number of slow movements.
9. Minimize the opening and closing of doors, as this can cause air disturbances, which could interfere with cabinet airflow.
10. After removing all materials, repeat decontamination of the interior surfaces. Never use a cabinet to store supplies or laboratory equipment inside the work surface.
11. The ABSL2 room lab animal tech will wipe down all work surfaces before leaving at the end of the day to ensure all surfaces are decontaminated.
12. It is recommended that the cabinet be left running continuously to ensure a clean working environment. In order to assure proper airflow the slotted front grill must be kept clean of debris at all times.
13. Once a month the Lab Animal Tech should remove the cabinet’s stainless steel work surface and front grill and clean out any debris that has fallen through the slotted front grill. The pre-filter located underneath the work surface should be inspected and cleaned.

**IV. NEEDED EQUIPMENT**

None

**V. SAFETY CONSIDERATIONS**

A. Never operate a cabinet while a warning light or alarm is on. These warning devices indicate a compromise of cabinet integrity. Be sure to correct the problem before continuing.

B. Be sure to wear proper protective equipment when utilizing disinfectants under a hood.

1. Many of the cage changing units have an electric lift that adjusts the work surface height to the user’s preference. Using this feature can reduce back strain, etc. from repetitive cage changing.

**VI. NEEDED DOCUMENTATION**

None

1. **Proper disposal of carcasses – All carcasses are removed by an approved Biohazardous Waste Disposal Vendor .**

DLAM and research personnel will be responsible for removing dead animals from the tubs in the housing area. The animals will be removed and placed in a small carcass bag. The bags will be sprayed down with Sporklenz and placed in red biohazard trash with a red biohazard liner for holding until transportation to the freezer where the carcasses are held until the BioHazard Waste vendor takes them.

1. **Transporting animals to upstairs labs**

Animals/caging must be placed inside a tyvek bag/cover for transport. Spray outside of bag lightly with sporeklenz before removal from ABSL2 suite. Carts used for transport must be wiped down and wheels sprayed with Sporeklenz prior to leaving the suite.

1. **Returning soiled caging from upstairs labs**

Return soiled caging (cages transported from ABSL2 to upstairs lab for use) to ABSL2 suite and place in red biobubble for autoclaving. Cover open top of stack/tub with lid to reduce potential exposure to pathogens. Spray down exterior surface of tubs with Sporklenz. Close red bio bubble.

**TRAINING VERIFICATION**

I have read and understand the procedures outlined in this policy.

Date:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Trainee (print name):\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Signature;\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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