



Office of Laboratory Animal Care Standard Operating Procedures

Biocontainment Husbandry – Animal Biosafety Level 2

1. **Background**

This document describes procedures and guidelines for animal husbandry and research work in the SDSU OLAC Vivarium Biocontainment Suite. ABSL-2 areas are accessible to authorized, trained personnel only. All others, including Facilities Services personnel, contractors, vendors, external collaborators must be accompanied by authorized OLAC, EH&S, or laboratory members. This document addresses husbandry, personnel safety, biosecurity, and entrance and exit procedures. It also addresses handling, processing and disposal of caging, waste, carcasses and other items exposed or possibly exposed to biohazardous agents in use in the suite.

2. **Responsible Individuals**

OLAC technicians, OLAC manager, Research Personnel

3. **Definitions**

ABSL-2: Animal Biosafety Level 2 builds upon the practices, procedures, containment equipment, and facility requirements of ABSL-1. ABSL-2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment.

Disinfectant: refers to the use of an approved and appropriate disinfectant for the appropriate contact time to achieve disinfection, as described in the BUA. In general, Cavicide is used in the ABSL2 animal facility and is effective against most agents with a three minute contact time.

4. **Materials**

- Disposable isolation gown (long pants required to be worn under gown. Shorts or skirts are prohibited)
- Shoe covers (open-toed shoes are prohibited)
- Head cover
- Face mask
 - Surgical mask if all work is being conducted in a biosafety cabinet
 - Filtering face piece respirator (aka N95 mask) if work is conducted on an open bench
- Eye protection

- Face shield, safety glasses, or prescription glasses if work is conducted in biosafety cabinet
- Face shield or safety glasses if work is conducted on an open bench
- Gloves
- Approved disinfectant
- Biohazard waste disposal bins
- Chemotherapy waste bins
- Autoclavable biohazard bags
- Microisolator cage sets including cage bottom, wire bar feeder, water bottle, cage card holders, cage cards and intact filter top
- Scraper
- Rodent diet
- Glue traps

5. **Documentation and Communication**

OLAC staff is responsible for completing animal health checks and the room log each day, and recording environmental parameters of maximum and minimum room temperatures/humidity, husbandry activity and relevant comments. Animal health, breeding management and compliance issues are emailed to designated lab personnel and OLAC veterinarians.

6. **Procedures**

Entry Procedures

All personnel entering the housing room must don the personal protective clothing listed below in the anteroom:

- Disposable isolation gown
- Head cover
- Face mask
 - Surgical mask if all work is being conducted in a biosafety cabinet
 - Filtering face piece respirator (aka N95 mask) if work is conducted on an open bench
- Eye protection
 - Face shield, safety glasses, or prescription glasses if work is conducted in biosafety cabinet
 - Face shield or safety glasses if work is conducted on an open bench
- Shoe covers
- 2 pairs of latex, vinyl, or nitrile gloves

Cage Changes

Room checks and environmental parameters are performed by OLAC and recorded as indicated in the SDSU OLAC SOP: [Daily Health Monitoring, Record Keeping, and Investigator Notification](#).

Cage changes are performed in a biosafety cabinet. Prior to cage changing, disinfect the biosafety cabinet and the outside of the cages to be changed. Arrange cage changing materials in the biosafety cabinet with a clean to dirty work flow. To ensure proper biosafety cabinet containment, do not overcrowd the space or cover vents with materials.

If bottoms only are to be changed:

- Transfer animals to the clean cage
- Add food as needed
- Transfer other cage components to the clean cage
- Stack all dirty caging neatly in the cabinet
- At appropriate intervals, use a scraper to dump soiled bedding from cages into the designated receptacle lined with an autoclavable biohazard bag under
- At appropriate intervals, either disinfect or bag cages for disinfection and remove from biosafety cabinet in order to continue cage changing without clutter (see “Cage Disinfection” below)

If wire bar feeder, water bottles and/or filter tops are to be changed:

- Transfer animals into complete clean cage sets
- Add food as needed
- Stack all dirty caging and equipment neatly in the hole in cabinet
- At appropriate intervals, use a scraper to dump soiled bedding from cages into the designated receptacle lined with an autoclavable biohazard bag
- At appropriate intervals, either disinfect or bag cages/equipment for disinfection and remove from biosafety cabinet in order to continue cage changing without clutter (see “Cage Disinfection” below)

Between each group of investigator and agent-defined mice:

- Disinfect biosafety cabinet by wiping down all work surfaces of the biosafety cabinet using with approved disinfectant
- Change outer gloves

Research Activities

All research activities must be done within the biosafety cabinet unless otherwise approved by the IBC. Samples and reusable surgical tools/equipment must be disinfected before removal from the biosafety cabinet.

If research activities are performed outside of the biosafety cabinet, an N95 mask must be worn by personnel. Samples and re-usable surgical tools/equipment must be disinfected before removal from the ABSL2 room.

7. Disinfection

Cage Disinfection

- Dispose of bedding and other disposable accessories as biohazardous waste (or chemo-waste if a hazardous chemical was used)
- Disinfect cages and re-usable accessories with appropriate disinfectant in the biosafety cabinet. Alternatively, cages and re-usable accessories can be bagged in autoclavable biohazard bags inside the biosafety cabinet, before they are taken to the autoclave for disinfection. Bagged caging is sprayed with the disinfectant and placed in a secondary container before removal from the housing room. They are then taken to autoclave room

for sterilization. The autoclave is tested monthly using appropriate sport strips or ampules to ensure proper sterilization

- For autoclave disinfection, cage bottoms, wire bar feeders, cage card holders and filter tops are autoclaved in bags using the dry cycle. Soiled used bottles are placed upright in intact rate cages with a microfilter top and autoclaved using the liquid cycle
- All appropriately disinfected reusable caging may be transferred to the cage washer for usual processing

Room Disinfection

- The floor is mopped with approved disinfectant using a dedicated mop bucket/wringer and mop kept in the ABSL2 suite. Discarded mop heads must be disposed as biohazardous waste when replaced.
- Used liquid disinfectant may be poured down the drain of the sink inside the ABSL2 suite

Research Sample/Equipment Disinfection

- Spray samples, reusable equipment and surgical tools, such as scissors, forceps, pipettors, pens, etc., with appropriate disinfectant for appropriate contact time for disinfection. Organic matter should be removed prior to disinfect. Ensure that all surfaces are sprayed. Place samples in a sealed, rigid, leak-proof secondary container. Disinfect exterior of secondary container before removal from ABSL2 room.
- Porous items that cannot withstand disinfection, such as papers, lab notebooks, cell phones, etc, must be placed in a sealed, non-porous container (such as a plastic sheet protector, Ziploc bag, etc) before entering the ABSL2 suite and may not be removed from the sealed, nonporous container while in the ABSL2 suite. Disinfect the nonporous container prior to exiting the room.

8. Exit Procedures

Caging, samples, and re-usable equipment/surgical tools

After removing outer pair of gloves, place disinfected items on a transfer cart in the ABSL2 anteroom then proceed with “De-gowning” below.

De-Gowning Procedure

1. Remove the outer pair of gloves and shoe covers and dispose of them in a biohazard bin in the housing room.
2. Exiting the housing room. Spray disinfectant onto the door handle and gloves and close the door to the ABSL-2 room
3. Aseptically remove disposable gown
 - a. With clean inner gloves, reach behind your neck and untie/tear disposable gown. As you remove the gown, flip it inside out and roll it up so that there is no contact with the exterior of the gown
 - b. Dispose of gown in biohazard trash in the ante room
 - c. Spray disinfectant on gloves
4. Aseptically remove hair and face protection

- a. With disinfected gloves, grip the edge of PPE and remove carefully so there is no unprotected contact with exterior of PPE
 - b. If PPE is reusable, spray with disinfectant and allow appropriate contact time
 - c. Discard disposable PPE in biohazard trash in anteroom
5. Aseptically remove inner gloves
 - a. Grip the exterior cuff of glove on one hand and remove glove by turning it inside out while peeling off.
 - b. With ungloved hand reach under the cuff of the remaining glove and flip inside out as you remove the glove, avoiding contact with the exterior of the glove
 - c. Discard used gloves in the biohazard trash in the anteroom
6. Wash hands before exiting the anteroom

Waste, Sharp, and Carcass Handling

- All trash or other solid waste generated in the housing room is placed in biohazard bags inside rigid secondary containers. The exterior of the full biohazard bags are sprayed with approved disinfectant for appropriate contact time prior to removal from the housing room and placed in a rigid secondary container to be taken to biohazardous waste collection site
- All Sharps are disposed of in appropriate sharps collection containers. When full the containers are sealed and the exterior is disinfected prior to removal from the housing room and placing in a rigid secondary container to be taken to biohazardous waste collection site.
- Carcasses are transferred to clear ziplock bags inside the biosafety cabinet. If OLAC has found the animal dead, the bag will be labeled with the PI and animal information. The exterior of the bag is disinfected prior to removal from the housing room. Bagged carcasses generated by the lab may be placed in a biohazard bag inside the carcass freezer located in the ABSL2 anteroom. OLAC will place bagged carcasses of animals found dead in the carcass refrigerator in the ABSL2 anteroom for the lab to pick up if tissues are needed. If the carcass has not been picked up within 24 hours, OLAC will transfer it to the carcass freezer. Carcasses in biohazard bags will be placed in a rigid container for transfer to the biohazard waste collection site.