WVU ACUC APPROVED STANDARD OPERATING PROCEDURE:
OLAR ABSL-2 Use in Animals

Agents designated as Biosafety Level 2 (BSL-2) are assigned by the Institutional Biosafety Committee (IBC) based on a risk assessment. The definition of ‘Risk Assessment’ as per Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th edition is as follows: ‘Risk assessment is a process used to identify the hazardous characteristics of a known infectious or potentially infectious agent or material, the activities that can result in a person’s exposure to an agent, the likelihood that such exposure will cause a Laboratory Acquired Infection, and the probable consequences of such an infection’.

Definitions:

Animal Biosafety Level 2 (ABSL-2) refers to animal use involving agents and conditions designated as such by the IBC. Although this document most commonly applies to mice, it is in place for all species that are handled at an ABSL-2 level.

PPE: Personal protective equipment; typically this means wearing a cap face mask, gloves, gown and shoe covers depending on procedures approved for the specific vivarium.

Rooms and areas designated as ABSL-2 will be subject to the following practices:

1. Biohazard labels will be posted on the doors of each ABSL2/BSL2 designated space without specific agents listed. The cages where infectious agents, including human cell lines, potentially exist must be labeled with a biohazard sticker (usually on the cage card) identifying the agent and the PI. The biohazard label or door signage will list appropriate PPE and safety precautions. If biohazard sticker affixed cages are brought to a lab, the lab door should have a biohazard sign with appropriate PPE for entry listed, as well as emergency contact information.

2. Workers wear PPE for allergen exposure as per standard OLAR barrier housing procedures. N-95 respirators are not required, unless prescribed by Occupational Health or the IBC based on a revised risk assessment. If an elevated risk assessment is established, (e.g. use of human pathogens spread by respiratory exposure), signage requiring N-95 or PAPR use and fit testing (required for N-95 use) prior to entry will be posted on the ABSL-2 door. At any time, workers have the option to wear N-95 respirators provided by OLAR. Contact the biosafety officer or OLAR for guidance, if questions arise.

3. Persons entering ABSL-2 areas that typically involve the use of human origin tissues or reagents must complete the following training:
   a. The BSL2 and Biosafety for animal use training modules on the CITI website every 2 years.
   b. Blood-borne pathogens training must be completed annually http://ehs.wvu.edu/biosafety/ibc-protocol-submissions-and-forms
4. If you are unsure of the status of a proposed agent, biological origin reagents to be used in animals, or procedures in regards to their biological classification as to risk, contact the Biosafety Officer (304-293-7157).

5. All standard OLAR housing practices to enter or exit any rodent holding or procedural room apply, including: appropriate training, PPE practices, sharps handling, proper biosafety cabinet use, appropriate waste disposal, ACUC protocol approval, etc.

6. Personnel using ABSL-2 designated animal rooms and procedural spaces and following ABSL-2 procedures will bag cages, PPE (use labeled biohazard containers) and biohazardous waste, and label all such bags as biohazardous with the PI and agent clearly identified. All bedding and dirty cages will be bagged using clear (internal use) autoclave bags by the person discarding the cage within the ABSL-2 room (PI, staff or OLAR) and OLAR staff will autoclave these cages prior to bedding disposal and cage washing. Biohazardous waste or disposables will be placed in a designated biohazardous waste container for autoclaving before disposal. Mixed biological and chemical hazardous waste will be disinfected by addition of disinfectant to the mix before addition to the chemical waste. Chemical hazardous waste is then disposed of. Contact Environmental Health and Safety for an appropriate disinfectant that is compatible with the chemicals involved.

7. A second alternative, in the event that the autoclave is down for repairs, and depending on the agent is to use wet disinfection in a biosafety cabinet in the ABSL-2 room, or on the dirty side of the cage wash. The OLAR Director will make this assessment to protect personnel.

8. If a spill of biohazardous materials occurs, spill response forms listing the steps for cleaning up a spill are posted in all ABSL-2 rooms. Spill clean-up materials are available in all ABSL-2 rooms. A biohazard incident report must be filled out and sent to the biosafety officer. Forms can be found on the WVU biosafety website, http://ehs.wvu.edu/biosafety/biohazardous-incident-reporting.

9. Between projects and each cage use, there will be regular and thorough decontamination of surfaces and equipment using an appropriate high-level (in date) disinfectant formulation provided by OLAR (SporGon, SporKlens, Clidox, paraformaldehyde or Virkon). The room will be disinfected before it is turned over to normal use from ABSL-2, and such a switch cannot be performed until Rodac or equivalent testing shows the room is rendered safe.

10. ABSL-2 necropsy procedures should be done in ABSL-2 rooms (whenever possible) inside a biosafety cabinet using BSL-2 practices. Contact OLAR if a deviation from this regime is required. Carcasses are double-bagged in the room, labeled with the agent and PI, disinfected and deposited directly in the designated biohazard container in the cooler for disposal. Biohazard bags for pick-up must be closed and double-bagged before transport.

11. All sample containers, instruments or other materials must be disinfected before removal from any ABSL-2 room.

12. HEPA filters are used in the anesthesia patient circuit (both at the patient and before the scavenging system or canister) for gas anesthesia equipment, and a log kept of hours of use. After 24 hours of use (or for the period designated on the brand of filter unit used), HEPA filters are replaced and discarded into the designated BSL-2 waste bin destined for autoclaving, before disposal.
13. Sharps will be disposed of appropriately. Any injury from a sharp object or infected animal will be treated immediately with disinfectant (scrub brush with disinfectant supplied in each room). If necessary, the person will be referred to Occupational Medicine or the Emergency room (if severe). First aid kits with disinfectant will be located in each hallway and disinfectant laden scrub brushes will be found in each ABSL-2 room. If the exposure involves suspected contact with a BSL-2 agent or ABSL-2 animal, contact the Biosafety Officer (304-293-7157). An EH & S incident report and a biosafety incident report must be filed as soon as possible (forms at OLAR entry and EH & S website).

14. Anesthesia or adequate restraint (and adequate prior animal handling training by the PI or OLAR) should be used when injecting or using sharps in contact with live animals.

15. All ABSL-2 holding rooms housing immunodeficient (e.g. SCID, XID, Beige, RAG knockout, Nude, Non-obese diabetic SCID gamma chain knockout (NSG)) animals will have all bedding/cages/water bottles and other components that contact animals autoclaved before room entry to protect these immunocompromised animals. Some of these animals may also receive antibiotic treated water or food as indicated.

16. Animals being transported from an ABSL-2 room are assumed to be infectious, and must be transported out of OLAR within double-sealed containment such as in a cage with a lid and an additional enclosure, such as a sealable plastic container or taped autoclave bag (only if short-term, so rodents do not risk suffocation or overheating). Covering the cage from the public is still necessary if the outer covering is not opaque. Only the inner container needs have a biohazard sticker during transport.

17. In large animal rooms where ABSL-2 procedures are required, the following practices apply:
   o All ABSL-2 practices apply as described above, including PPE, door signage and cage or enclosure identification (PI and agent).
   o Depending on the agent, animals involved in ABSL-2 work may be housed with others. In such instances, the room is treated as ABSL-2, and the non-infected animals are accessed first. Contact the OLAR director if there are questions related to the cage changing order.
   o In such instances, where possible, the animals will receive clean caging right before the BSL-2 agent is delivered to minimize handling. When cage changing is to occur, large animal caging or racks are wrapped in bulk, and all wheels are sprayed with high-level disinfectant before leaving the room. If a bulk autoclave is available, then the cage is autoclaved before cage washing. If no autoclave is available, the rack or cage is sprayed with disinfectant in the room (including the wheels), and then transported to the dirty cage wash area and thoroughly disinfected again with a high-level disinfectant before entering the cage washer. All dirty bedding/disposables will be disinfected chemically, or bagged and autoclaved as appropriate.
   o Added procedures for safe animal handling may apply as guided by the IBC and OLAR.

Additional considerations for animal care:

A. Immune competent mice + human origin reagents or cells, including established human tumor cell lines of human origin with unknown human pathogen status:
   a. standard ABSL-2 procedures as above.
b. biohazard stickers on cages for the protection and education of OLAR and other research personnel.
c. IBC approval is required

B. Any mice + **known** human pathogens (as designated by the BMBL).
   a. ABSL-2 procedures as above.
   b. biohazard stickers on cages at all times.
   c. IBC may add additional requirements for handling or PPE based on risk assessment.
   d. All pathogens at the BSL-2 or ABSL-2 level or greater require IBC approval before work can start. Work with agents at a level above ABSL-2 requires a biocontainment facility and practices specifically designed for this purpose.

C. Any mice + human origin reagents with risk of murine pathogens, (including human cells passaged in mice, or in contact with mouse origin reagents).
   a. ABSL-2 procedures as above.
   b. biohazard stickers are not required on cages.
   c. testing of cell lines for murine pathogens is required. Frequency of testing and requirements for eliminating pathogens from cell lines are to be determined pending discussion with the OLAR veterinarians.

**Testing Requirements for Human or Murine Origin Biologicals:**

**Any human cells (cell lines, blood or primary tissue) or human origin reagents:** Samples will be treated as if they are infectious, so no additional testing is required for human agents. However, testing is available and encouraged. However, there will be a requirement to test human cell or reagents for *Mycoplasma* spp. that may infect rodents. **NOTE:** Principal Investigators are responsible for ensuring their human cells, cell lines or human origin reagents are not infected with mouse pathogens, and can contact OLAR for screening options.

**Any murine cells or murine origin reagents:** Testing of cell lines for murine pathogens is required. Frequency of testing and requirements for eliminating pathogens from cell lines will be addressed by OLAR by working with the PI.

**In house generated murine origin tissue, reagents or blood:** Procedures will be used based on the source and health status of the donor and recipient mice. Contact OLAR if you have questions.

Approved by IBC