The scope of this standard operating procedure (SOP) pertains to Animal Biosafety Level 2 (ABSL2) housing for animals inoculated with Risk Group 2 microorganisms. ABSL3 containment is excluded and addressed by the lab specific standard operating procedures.

All biological hazards utilized in vertebrate research must be documented in an Institutional Animal Care and Use Committee (IACUC) approved protocol. Biological hazards requiring Biosafety Level 2 containment must be documented in an approved Environment Health and Safety (EHS) Lab Safety Plan, Schedule F. Research involving the use of recombinant or synthetic nucleic acids must be documented in an Institutional Biosafety Committee approved Environment Health and Safety Lab Safety Plan, Schedule G.

The Principal Investigator is responsible for evaluating the risks associated with the proposed research and obtaining the necessary approvals prior to conducting the research. The Principal Investigator is responsible for notifying research personnel as well as animal husbandry staff of the potential hazards they may be exposed to while carrying out their duties. The hazard assessment must include potential hazards during the following activities:
Culture and/or preparation of the inoculum
Transport of inoculum or inoculated animals
Animal inoculations
Husbandry activities including cage changing and disposal of bedding
Waste disposal

This SOP is directed specifically to the husbandry activities including cage changing, bedding and waste disposal of ABSL2 contaminated articles and PPE within DLAM controlled ABSL2 space.

**Animal Biosafety Level 2 (ABSL2)**
Animal Biosafety Level 2 (ABSL2) builds upon the practices, procedures, containment equipment and facility requirements for ABSL1. ABSL2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment.

For further guidance refer to:
- EHS Lab Safety Manual – Chapter 5: Vertebrate Animal Biosafety Level Criteria and Summary of Recommended Biosafety Levels for Infectious Agents
- Biosafety in Microbiological and Biomedical Research, Edition 5, Section 5: Vertebrate Animal Biosafety Level Criteria for Vivarium Research Facilities

**Biological Hazards within the Scope of this SOP**
Risk group 2 agents within the scope of this SOP are known to consistently cause moderate to severe disease in healthy adult humans for which vaccines or therapeutics are often available.

For guidance refer to:
- NIH Guidelines Appendix B. Classification of Human Etiological Agents on the Basis of Hazard
- American Biological Safety Association Risk Group Classification Database

**Viral Vectors**
Based on a risk assessment, animals inoculated with commonly utilized replication deficient viral vectors developed from risk group 2 viruses (retroviruses, lentiviruses and adenoviruses) will be housed initially at ABSL2 and downgraded to ABSL1 following a 72 hour shedding period and subsequent change to clean caging. The animal cage change may be performed by either DLAM husbandry personnel or members of the research staff, as established by mutual arrangement between DLAM and PI prior to study initiation. Animals inoculated with viral vectors derived from viruses other than those specified above will require permanent ABSL2 housing unless otherwise specified by a risk assessment to be conducted by the EHS biological safety section.

**Adeno-Associated Virus (AAV)**
Adeno-Associated Virus (AAV) is classified as a risk group 1 virus. As per NIH Guidelines Appendix B-I, recombinant AAV encoding non-toxigenic or non-tumorigenic genes is classified as a risk group 1 organism. As such, it requires BSL-1 containment and animals inoculated with such viruses may be housed at ABSL1, excluding them from the scope of this SOP.
Attenuated Microorganisms with Established Clearance from Animal Hosts
In some well-established models involving the infection of immune competent animals, the host immune response is able to reproducibly eliminate infection by a certain date post infection. In such models, the PI is responsible for informing DLAM personnel of the nature of the risk and indicating on the biohazard card the date by which the animal will no longer pose a biohazard. Following the shedding period, the Investigator will be responsible for transferring the animal to clean caging and removing the biohazard card.

ABSL2 Procedures
The following procedures must be followed when working at ABSL2. The procedures may need to be modified for animals other than rodents. Contact DLAM for information regarding other species.

1. Notification and Signage
   Laboratory personnel must provide advance notice to DLAM personnel (AOD, Facility Manager/Supervisor or Veterinarian with oversight of ABSL2 areas) prior to conducting experiments requiring ABSL2 containment in DLAM facilities. The Principal Investigator is responsible for ensuring DLAM personnel are made aware of the risk associated with the inoculated animals. Laboratory personnel must label each cage appropriately with “Biological Hazard” cage cards.
   • Within the Investigator’s laboratory, cage labels can take any form that is recognizable to all laboratory workers.
   • All DLAM or satellite ABSL2 facilities require an ABSL-2 placard to be posted at the entrance to the room.
   • For animals housed in DLAM-managed areas:
     o At least three business days prior to animal inoculations, the laboratory will notify the DLAM Facility Manager/Supervisor of the inoculation schedule and route of administration. The work cannot begin until the laboratory has obtained confirmation that their scheduling notification has been received and can be accommodated.
     o An “ABSL-2 Animal Isolation Information” form must be posted on the cubicle (if utilized) or posted on the door (if no cubicle is utilized). This form can be obtained from the Facility Manager/Supervisor, AOD, or Veterinarian responsible for ABSL2 housing and must be completed with the IACUC protocol number, name and contact information of the individual(s) responsible and specific agent(s) to be used. DLAM will complete the assignment of ABSL2 space upon receipt of this form.
     o When inoculated animals are returned to their cages, orange “Biological Hazard” cage cards (supplied by DLAM) must be placed on each cage. Information on these cards should contain the PI name, agent being used, route of administration, and inoculation date(s).

2. Engineering Controls
   • Cages will be covered with micro-isolator lids and/or will be maintained either on an individually ventilated cage rack or in static micro-isolator caging.
   • Cages will be opened (including for cage-changing, animal care or experiment-related reasons) in a biological safety cabinet, or a chemical fume hood.
• For cage changing of ABSL-2 cages, or at termination of animals, the dirty cages will have the lids replaced **after removing all animals**, and the cages labeled for “autoclave out” only. **All individuals must assure that ALL animals are removed from cages before placing the cage on the dirty cage rack.** This will be documented by two DLAM husbandry individuals per SOP “Checking Soiled Cages Before Autoclaving”. Cages will then be autoclaved prior to opening again. After autoclaving, cages can be processed as normal dirty cages because all ABSL-2 level infectious agents are killed by autoclaving.

3. **Personal Protective Equipment**

   Employees must wear the following personal protective equipment for handling animals, cages and bedding:
   • Shoe Covers and Closed-toe shoes
   • Safety Glasses, Goggles, or Face Shield
   • Face Mask
   • Gloves
   • Bonnet or Cap
   • Disposable Lab Coat, Coveralls or Tyvek Suit
   • Safety glasses are to be worn at all times when opening cages, moving cages from eye level or above and handling bedding (filling cages, emptying cages) unless done inside a biological safety cabinet or a chemical fume hood.

4. **DLAM ABSL2 PPE and Waste Disposal Policy**

   For all rodent DLAM managed ABSL2 areas, the following PPE must be placed in autoclave bags upon exiting the ABSL2 area and be autoclaved before disposal. If the facility does not have the capability of autoclaving PPE/trash from the ABSL2 area, then a Stericycle box should be provided and included for pickup each week with animal carcasses.
   • 2nd pair of shoe covers in GM, shoe covers in other facilities
   • 2nd pair of gloves in GM, gloves in other facilities
   • Sleeves, if required
   • If there is a splash/spill (liquid/bedding), then face, and eye protection must be replaced or decontaminated.

   For all large animal DLAM managed ABSL2 areas, all PPE must be placed in autoclave bags upon exiting the ABSL2 area and autoclaved before disposal. If the facility does not have the capability of autoclaving PPE/trash from the ABSL2 area, then a Stericycle box should be provided and included for pickup each week with the carcasses.

5. **Work Practices**

   • Gloves must be changed when they become torn or obviously contaminated with excreta AND before handling animals in other experimental groups. Uninfected or naive control cages should be handled prior to infected cages in experimental groups.
   • Employees will wash hands after removing gloves.
   • Safety glasses, after use, will be disinfected, stored in a clean place, and reused.
• The husbandry technician will change cages following the procedures outlined in the “Working with Animals in a Biosafety Cabinet or Changing Station” SOP.
• All bedding, food and water present in a rodent cage within 3 days of dosing for replication deficient vectors, or for the duration of housing at the ABSL2 level will be considered contaminated until the cage unit is autoclaved. These cages will be autoclaved after removal of animals, as described above.
• At end of study, all cage cards (RFID, Vet Care) will be placed in the plastic sleeve on the inside of the door of the ABSL2 room, and will be disinfected by a designated DLAM employee prior to distribution to Vet Services or DLAM Administration.
• Decontamination of cage changing areas after each use will consist of cleaning up spilled bedding, placing it into a dirty cage, and spraying the changing surfaces with an approved disinfectant following the procedures outlined in the “Working With Animals in a Biosafety Cabinet or Changing Station” SOP.
• Euthanasia of ABSL2 infected animals will be by approved methods described in the IACUC protocol. DLAM installed euthanasia stations are present in all ABSL2 cubicle rooms, or adjacent ABSL2 placarded procedure rooms.
• As with any animal bite or scratch, employees bitten or scratched by animals exposed to hazardous agents are expected to notify their supervisor immediately. The employee should also note the specific biological agent(s) and go directly to the University Employee Occupational Health Clinic. For after-hours exposures, please call UEOHC at 919-966-9119.
Biological Hazard

Biological Agent: _____________________________

P.I. Name: _____________ IACUC Protocol#: _________

Route of Administration (Please Circle):

Tail Vein    Intra Nasal
IP           Intra Cranial
Subcutaneous Oral
Other:

Inoculation Date(s): ________________________
A. **Investigator Information**

IACUC Protocol Number ________________________ Investigator ______________________

Last First

Department _________________________________

Contact Person _____________________________

Telephone Number (lab and emergency) _________________________________

Species _________________________________

B. **Reason(s) For Isolation**

Biosafety hazard – specific agent(s)

__________________________________________

__________________________________________

C. **Personal Protective Equipment to be Worn When Working With These Animals**

Cap/ Bonnet ☒ Gown/ Tyvek Suit ☒ Mask ☒ Gloves ☒ Shoe covers ☒

Tyvek Sleeves ☐ Goggles/Safety Glasses/Face shield ☒

Other ☐ ____________________________ (specify)

D. **Handling of Animals and Equipment**

Animals ☒ Incinerate _________________________

Feed ☒ Autoclave in/out ______________________

Bedding ☒ Autoclave in/out ______________________

Cages ☒ Autoclave in/out ______________________

Water Bottles ☒ Autoclave in/out _____________

Other ☐ _________________________________ (specify)

**For DLAM Use Only**

<table>
<thead>
<tr>
<th>Building</th>
<th>Cubicle #</th>
<th>Assigned By</th>
<th>Date</th>
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<td>J Nielsen</td>
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Precautions

Potential Human pathogens – wear appropriate PPE

To request new or updated forms call Dr Nielsen at 843-6536 or e-mail Judith_nielsen@med.unc.edu.