1. SCOPE

This Standard Operation Procedure (SOP) covers all recombinant viral work involving research rodents. Examples of viral vector systems covered by this SOP are: ectropic (with hot gene), amphotropic, and pantropic (with hot gene) retroviral systems; Adeno-Associated Virus (AAV); and Adenovirus (AdV).

2. PURPOSE

This document will cover the basic requirements for administering the recombinant virus, identification of animal cages, husbandry requirements, and waste handling requirements.

3. BACKGROUND

Viral Vector systems, and subsequent recombinant virus, have become powerful tools for various aspects of research. With the availability of kits, and companies propagating the recombinant virus for users, there has been a rapid increase in the usages of these tools.

Designers of these molecular tools have imbedded certain safety features to help protect the users, and any patients/animals being treated with the recombinant virus. Some of these features include the removal of all unnecessary genes. This helps to eliminate the possibility that the virus will recombine to form a functional virus. Additionally, most feature a self-inactivating sequence, which prevents the virus from replicating. This means that the virus will infect a cell, but the virus will not go on to replicate in that cell, it supplies the gene of interest and that is all. Finally, the required genes for producing the recombinant virus are spread over several plasmids. Again, this makes it unlikely for accidental recombination of the genes into a functioning virus.
Due to all of these safety features, animals infected with recombinant viruses may be downgraded from Animal Containment Level 2 (ACL2) to ACL1 after the shedding period or the first cage change - whichever is longer.

4. RESPONSIBILITY

It is the responsibility of each Principle Investigator to:

1. Ensure that all students/staff have received proper training in handling animals and recombinant viruses. This includes the UBC Animal Care Courses, UBC Biosafety Course, procedure specific training, and site-specific orientation*.

2. Ensure that all Institutional and Federal Approvals are obtained. This includes but is not limited to Animal Ethics, Biosafety Permits, Importation Permits, and Transfer Records.

3. Ensure that all Animal Facility Technicians are made aware of the Viral Vector Systems being used, and their risks associated to the virus system (shedding period and route of exposure) and the genetic modifications (gene of interest, knock-down, or overexpression).

4. Ensure that all students/staff have access to appropriate personal protective equipment (PPE), engineering controls, and administrative controls. Ensure that all staff is utilizing this equipment.

It is the responsibility of Animal Facility Managers to:

1. Ensure that all Animal Care Technicians have received proper training in the handling and husbandry of animals and recombinant viruses. UBC Animal Care Courses, UBC Biosafety Course, procedure specific training, and site-specific orientation*. If there is an expectation that staff will be doing more than basic health checks and husbandry procedures, then they must be trained to the same level as the research staff/students.

2. Ensure that all staff knows where to find information on the specific projects of a given room.

3. Ensure that all staff has access to appropriate PPE, engineering controls, and administrative controls. Ensure that all staff is utilizing this equipment.

It is the responsibility of ALL research students/staff and animal care staff to:

1. Ensure they follow the SOPs and training that has been provided.

2. Report any incidents, exposures, and near misses to their supervisor.

3. Ensure they: wear the provided PPE, use the provided engineering controls, and follow the provided administrative controls.

*Site Specific Orientations – a review of specific facilities, equipment, systems, and procedures for the particular animal unit that the PI is using. This is most often performed by Animal Care Workers.
5. REFERENCES AND DEFINITIONS

Canadian Biosafety Standards and Guidelines
Canadian Council on Animal Care
National Institute of Health: Recombinant DNA Advisory Committee

6. MATERIALS/ EQUIPMENT

- Labels
- Shoebox; Micro-isolator or Ventilated caging
- Recombinant Virus Information Sheet
- Needles and Syringes (if applicable) - use safety needles wherever possible (See RMS Guidance Document: UBCV-RMS-ORS-GDL-008)
- Biosafety Cabinet (BSC) and/or Cage Change Station (CCS) (IBC-SOP-002 Safe Use of Biosafety Cabinets)
- Anesthetic Gas Set-up including a Scavenging system
- Restraints (stereotaxic equipment, tubes, or other approved means)
- Decontaminant – all of the above mentioned recombinant viruses are susceptible to: 5000-10 000ppm Sodium Hypochlorite (5-10% Bleach); Virkon; and Phenokil. Follow what has been approved in the Biosafety Permit.

7. PERSONAL PROTECTIVE EQUIPMENT

The PPE required will depend on the type of facility in which the work will take place, and the route of exposure from the recombinant virus (i.e. inhalation, ingestion, etc).

NOTE: All students/staff who will be wearing an N95 respirator must be fit tested annually. Please contact Occupational & Preventative Health for an appointment.

PROCEDURE

Before work begins, the Hazard Information Sheet must be provided to the facilities housing the animals that will be infected with the recombinant virus.

Clearly relay the shedding period for the recombinant virus. If this is unknown, then provide justification in your UBC Biosafety Permit as to when the animals may be downgraded from ACL2 to ACL1.
Preparation of Recombinant Virus

PPE – Full loose fitting pants, full covering shoes, lab coat, minimum of 1 pair gloves (2 pairs of gloves are recommended, and required for lentivirus).

1. Unless special approval has been obtained, this work should occur in the Principal Investigator’s in vitro laboratory.
2. Aliquots of rVirus may be brought to the animal facility for all spaces that have access to a Biosafety Cabinet and an approved procedure room. Approvals come from Risk Management Services.
   If there is no access to a Biosafety Cabinet and approved procedure room then the virus must be pre-loaded into syringes (follow RMS Guidance Document: UBCV-RMS-ORS-GDL-008) and carried in a secondary container to the animal facility.
3. If aliquots are stored in, or brought to, the animal facility then the needle/syringe must be filled in a Biosafety Cabinet.
4. For procedures that involve exposing the animal via a different method than injection, please follow the ACC approved methods and equipment. This may include inhalation models and oral gavages.

Administration of rVirus

PPE (will depend on the classification of the space being used) – Full loose fitting pants, full covering shoes, lab coat, minimum of 1 pair gloves (2 pairs of gloves are recommended, and required for lentivirus).

1. Prepare the animal by the method approved in the Animal Ethics Approval.
2. Ensure the anaesthetic gas and scavenging systems are set-up appropriately.
3. Wherever possible exposures should take place in a Biosafety Cabinet.
4. Perform exposures by the method approved in the Animal Ethics Approval.
5. Dispose of any needles into biohazardous sharps waste (needles are not to be re-used); dispose of any consumables into RG1/RG2 waste; and pre-treat any reusable consumables (e.g. glassware) with decontaminant before it is taken to the cage wash area.
6. Decontaminate the work surfaces with the appropriate and approved decontaminant. This Dutch Disinfection Database will help determine the appropriate chemical.

Identification of Cages

1. All animals are to be housed in containment caging, either a micro-isolator cage or a ventilated caging system. If shoebox caging is the only option, then either a testable
HEPA filter enclosure (approved by Risk Management Services) must be used or the requirements for a “Large Animal Suite” must be followed, as containment will occur at the room level.

2. Researchers are responsible for labeling the cage cards with a rVirus sticker at the time of administration. The date of the exposure, shedding period, and initials of the researcher must also be included.

3. Ensure the exposure is documented on the facility notification system (e.g. whiteboard or database).

4. The exposure must also be noted in the ACC approved monitoring or procedure logs.

Monitoring and Health Checks during the Shedding Period

PPE (will depend on the classification of the space being used) – Full loose fitting pants, full covering shoes, lab coat, minimum of 1 pair gloves (2 pairs of gloves are recommended and required for lentivirus).

1. **All students/staff responsible for performing monitoring and health checks must ensure that they follow CL2 procedures until the shedding period is over or the first cage change, whichever is longer.**

2. If cages need to be opened prior to the first cage change/shedding period, then double gloves must be worn and the cage taken to either a CCS/BSC before they are opened.

3. Outer gloves should be checked regularly for damage, and disinfected between handling cages.

4. Any bedding, water bottles, domes, or other objects that have been in contact with the INSIDE of the cage must be autoclaved prior to entering the regular cage washing procedure.

5. Any animal that has been euthanized prior to the first cage change/shedding period must be double bagged, place in the freezer, and labeled as CL2 pathological waste.

Cage Changing during the Shedding Period

PPE (will depend on the classification of the space being used)

**Set-up**

1. The Cages are to be changed one at a time and gloved hands are disinfected between each cage change. The “dirty” cage and the “clean” cage must be no less than 12 cm apart at all times.
2. During the cage exchange process continually check outer gloves for signs of damage (i.e. puncture). If damaged, gloves must be changed immediately.

3. Place the following items in the CCS/BSC and spray the outside of all items with disinfectant prior to putting them inside:
   a. Complete cage units
   b. A container of sterile water bottles filled
   c. A container of food
   d. An appropriate bag for RG1/RG2 waste. Label which room it is coming from with marker pen.
   e. To prevent leaks and breakage during storage or transportation, double bagging with another clear autoclave bag is required.

4. Place cages inside CCS/BSC. Remove the filter top and place inside up on the work surface.

Procedure - Follow normal cage changing procedures with the following additions:

1. If a dead animal is found, follow the site SOP; however the bag must be identifiable with a biohazard symbol, so that everyone knows the contents have not been decontaminated or autoclaved.

2. Place clear RG1/RG2 autoclavable biohazard bags in the BSC/CCS and as the cages are exchanged place used cages in it.

3. Discard food into soiled cage bottom after moving animals to the new cage.

4. Water bottles are placed in the clear autoclavable biohazard bag.

5. Bags must be taped shut with autoclave tape (wrap tape 2-3 times around the twisted end of the bag). The bag must be sprayed on all surfaces with decontaminate before exiting the BSC/CCS.

6. Bag complete sets/cage parts in clear autoclavable RG1/RG2 waste bags for removal to dirty cage processing. Mark the bags with a note that these bags need to be autoclaved prior to going through the cage wash.

Completion

1. Check to ensure all cages have been changed and that no water bottle has started leaking (look for discoloration of bedding)

2. All RG1/RG2 waste bags with dirty bedding, cages, water bottles, lids, and wire lids must be contained inside clear autoclave bags and taped shut prior to leaving the CCS/BSC.

3. Mop head must be soaked in appropriate decontaminate for 24 hours after use in housekeeping closet. Label bucket with date, contents, and initials.
4. If for any reason this procedure cannot be followed, immediately contact the facility manager.

Post Shedding Period

Animals may be downgraded to Animal Containment Level 1 once the shedding period has passed. Perform a cage change (as described above) and then transfer the cages to an ACL1 area.

Cage labels should have the rVirus sticker removed or crossed off.

Waste and Handling Procedures will revert to the standard clean animal procedures.

8. REVIEW AND RETENTION

This SOP is reviewed annually or whenever deemed necessary by the responsible departmental representative in Risk Management Services.

9. DOCUMENT APPROVAL SIGNATURES

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APPENDIX A: CDM SPECIFIC REQUIREMENTS

CDM specific details for SOP for Recombinant viral Work in Animal Facilities

- All cages must have RED BIOHAZARD STICKERS on the top of the cage card.
- The animals must be placed in a clean cage, with full food and new water bottle immediately after exposure.
- “Shedding” refers to the period of time after exposure to the recombinant agent and until such time as the recombinant agent is no longer being excreted from the rodent. Shedding for AV, AAV and Lentivirus is up to 72 hour after exposure.
- Do not cage change during “shedding” unless necessary – e.g. flood or sick animal
- Do not use automatic watering (valves) on cages during the shedding period. Automatic watering may be used after the shedding period and after the first cage change. If an Ehret or Allentown water bottle needs to be changed during the shedding period/before the first cage change, the cage must be placed in the BSC/CCS.
- After the first cage change after the shedding period the rodents and cages are considered CL1
- For the first cage change the filter top cage lids are only taken off the cage in a cage changing station (CCS) or BSC
- The first cage change after the shedding period must be a complete cage change, with new food/cage/bottle given.
- All CDM staff and researchers must wear a cap, N95 mask, gown and two pairs of gloves when actively working with animals that are exposed.
- After the first cage change of recombinant cages, or if the cage needs to be opened during the shedding period, the CCS/BSC/cart or counter top and all exposed supplies/materials must be thoroughly disinfected or disposed of BEFORE leaving the room and before changing CL1 cages that may also be in the room. If able, change CL1 cages prior to the recombinant ones on the same day.
- During the shedding period and until after the first cage change do not collect dirty bedding for sentinel exposure from these cages.