Recombinant Viral Work in Animal Facilities

SCOPE

This Standard Operation Procedure (SOP) covers all recombinant viral work involving research rodents. Examples of viral vector systems covered by this SOP are ecotropic, amphotropic, and pantropic retroviral systems; Adeno-Associated Virus (AAV); and Adenovirus (AdV). See Appendix A for viral system specific information.

PURPOSE

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

BACKGROUND

Viral Vector systems and subsequent recombinant viruses 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.

Determination of containment requirements is project dependent, as it depends upon:

- the safety features of the specific viral vector used.
- the animal species infected.
- the genetic cargo delivered.

In some cases, with approval from the UBC Biosafety Committee, animals treated 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.

RESPONSIBILITY

It is the responsibility of each Principal Investigator to:

- 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*.
- 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.
- Ensure that all Animal Facility Technicians are made aware of the Viral Vector Systems being used, the genetic modifications (gene of interest, knock-down, or overexpression), and the risks associated with the virus system (shedding period and route of exposure).
- Ensure that all students/staff have access to appropriate personal protective equipment (PPE), engineering controls, and administrative controls. Ensure that they are utilizing these control measures properly.

It is the responsibility of Animal Facility Managers to

- 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*.
- 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.
- Ensure that all Animal Facility Technicians are made aware of the Viral Vector Systems being used, the genetic modifications (gene of interest, knock-down, or overexpression), and the risks associated with the virus system (shedding period and route of exposure).
- Ensure that all students/staff have access to appropriate personal protective equipment (PPE), engineering controls, and administrative controls. Ensure that they are utilizing these control measures properly.

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

- Ensure they follow the SOPs and training that has been provided.
- Report any incidents, exposures, and near misses to their supervisor.
- Ensure they wear the provided PPE, use the provided engineering controls, and follow the provided administrative controls.

REFERENCES

Canadian Biosafety Standards and Guidelines

Canadian Council on Animal Care

National Institute of Health: Recombinant DNA Advisory Committee

MATERIALS/EQUIPMENT

- Labels
- Shoebox; Micro-isolator or Ventilated caging
- Recombinant Virus Information Sheet
- Needles and Syringes (if applicable) - use safety needles wherever possible (See *BIO--GDL-008: Handling and Disposing of Needles*)
- Biosafety Cabinet (BSC) and/or Cage Change Station (CCS) (see *BIO-SOP-002 Safe Use of Biosafety Cabinets*)
- Anesthetic Gas Set-up including a Scavenging system
- Restraints (stereotaxic equipment, tubes, or other approved means)
- Decontaminant – disinfectant that specific recombinant virus is susceptible to: Follow what has been approved in the Biosafety Permit.

PERSONAL PROTECTIVE EQUIPMENT (PPE)

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 Biosafety Permit information for the project must be provided to the facilities housing the animals that will be infected with the recombinant virus.

Clearly relay the **shedding period**[1] 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.

[1] Shedding period is the time during which an animal or person may excrete a biological agent that has been administered. Excretion depends on the agent and animal, but maybe in urine, feces, sweat, saliva, nasopharyngeal fluids, and blood. Shedding period

begins at administration of a biological substance and ends when the likelihood of excretion viable agent is passed. This is estimated based on published estimates for each biological agent.

For studies where the administration of a biological agent requires CL2 containment initially but ongoing holding in CL1 containment may be acceptable, the shedding period defines the length of the CL2 housing and handling period. Note that cage changing is also required prior to transfer to CL1.

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).

- Unless special approval has been obtained, this work should occur in the Principal Investigator's in vitro laboratory.
- Aliquots of rVirus may be brought to the animal facility that has access to a Biosafety Cabinet and an approved procedure room. Approvals for rVirus acquisition and handling come from the UBC Biosafety Committee.
- If there is no access to a Biosafety Cabinet and approved procedure room then the virus must be pre-loaded into syringes and carried in a secondary container to the animal facility.
- If aliquots are stored in, or brought to, the animal facility then the needle/syringe must be filled in a Biosafety Cabinet.
- Use of syringe and needle must follow *SRS Guidance BIO--GDL-008: Handling and Disposal of Needles.*
- All procedures that involve administering substances to animals must follow the ACC approved methods and equipment. This includes injection, inhalation, implantation and oral gavages.

Administration of rVirus

PPE (will depend on 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).

- Prepare the animal by the method approved in the Animal Ethics Approval. If anaesthetic is required by the ACUP,
    - ➢ Ensure the anesthetic gas and scavenging system is set-up appropriately.
    - ➢ Wherever possible exposures should take place in a Biosafety Cabinet.
- Perform exposures by the method approved in the Animal Ethics Approval.
- 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.
- Decontaminate the work surfaces with the appropriate and approved decontaminant.

Identification of Cages

- 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 Safety & Risk Services) must be used or the requirements for a "Large Animal Suite" must be followed, as containment will occur at the room level.
- Researchers are responsible for labeling the cage cards with an rVirus sticker at the time of administration. The date of the exposure, shedding period, and initials of the researcher must also be included.
- Ensure the exposure is documented on the facility notification system (e.g. cage level labeling, whiteboard or MOSAIC).
- 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).

- 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.
- 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.
- Outer gloves should be checked regularly for damage and disinfected between handling cages.
- 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.
- Any animal that has been euthanized prior to the first cage change/shedding period must be double bagged, labeled as CL2 pathological waste, and placed in the freezer.

Cage Changing during the Shedding Period

PPE will depend on the classification of the space being used.

Set-up

- Spray the outside of the following items with a disinfectant prior to putting them inside the CCS/BSC:
  - Complete cage units
  - A container of filled sterile water bottles
  - A container of animal feed
  - Clear RG1/RG2 autoclavable biohazard bags or any other autoclavable bag used at the facility for RG1/RG2 waste. Label which room the waste is coming from with a marker pen.
  - To prevent leaks and breakage during storage or transportation, double bagging with another autoclave bag is recommended.
- Place cages in the CCS/BSC. Remove the filter top and place it on the work surface.
- The cages are to be changed one at a time and gloved hands are disinfected between each cage change. Maintain some distance between the “dirty” cage and the “clean” cage.
- During the cage exchange process, continually check outer gloves for signs of damage (i.e. puncture). If damaged, gloves must be changed immediately.

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

- Dead animal must be handled as a RG2 waste. If a dead animal is found, follow the site SOP. The bag must be identified with a biohazard symbol, so that everyone knows the contents have not been decontaminated or autoclaved.
- Move animals to the new cage. Ensure that 100% of the soiled cages (including cage, bedding, water, food, enrichment devices like igloos, etc.) are bagged and labeled appropriately for autoclaving prior to processing for reuse.
- 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 decontaminant before exiting the CCS/BSC.
- Animal facilities have a specific procedure for cage changing. Refer to the facility-specific requirement.

Completion

- Check to ensure all cages have been changed and that no water bottle has leaked (look for discoloration of bedding).
- Mop head must be soaked in appropriate decontaminate for 24 hours after use in the housekeeping closet. Label bucket with date, contents, and initials.

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

REVIEW AND RETENTION

This SOP is reviewed annually or whenever deemed necessary by the UBC Biosafety Committee or the UBC Biosafety Office.

Appendix A: CONTAINMENT GUIDELINES BY VECTOR TYPE

Viral Vector	Risk Group	Containment		Shedding Period*	Operational Requirement**	Disinfectant	Remark
		in vitro	in vivo				
Adenovirus	RG2	CL-2	ACL-2	7 days	The adenoviral vector must be administered to animals under ACL-2 containment. Animals must be housed under ACL-2 containment for at least 7 days unless data is provided to justify a downgrade of containment in less than 7 days	Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach). Minimum 20 minute contact time	For first-generation vectors or infection of animals containing human cells or tissues, ACL-2 containment may be required for longer periods
Adeno-associated virus WITH helper virus	RG2	CL-2	ACL-2	NA – may not be moved to ACL1	AAV with helper virus must be administered to animals and animals must be housed under ACL-2 containment.	Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach). Minimum 20 minute contact time	
Adeno-associated virus NO helper virus	RG1	CL2	ACL-1	7 days		Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach). Minimum 20 minute contact time	AAV must be packaged under CL-2 due to use of HEK293 cells; once packaged, AAV may be handled at CL-1

Viral Vector	Risk Group	Containment		Shedding Period*	Operational Requirement**	Disinfectant	Remark
		in vitro	in vivo				
Murine leukemia Virus (Ecotropic)	RG1	CL1	ACL-1	2 days		Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach). Minimum 20 minute contact time	
Murine Leukemia Virus (Amphotropic)	RG2	CL-2	ACL-2	7 days	MLV must be administered to the animal under ACL-2 containment. Animals must be housed under ACL-2 containment for at least 7 days unless data is provided to justify a downgrade of containment in less than 7 days	Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach). Minimum 20 minute contact time	
Lentivirus	RG2	CL-2	ACL-2	7 days	Lentivirus must be administered to the animal under ACL-2 containment. Animals must be housed under ACL-2 containment for at least 7 days unless data is provided to justify a downgrade of containment in less than 7 days.	Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach). Minimum 20 minute contact time	Second generation or 3-plasmid lentivirus systems should be generated and used at CL-2 with the CL-3 operation procedure

Viral Vector	Risk Group	Containment		Shedding Period*	Operational Requirement**	Disinfectant	Remark
		in vitro	in vivo				
Herpes Virus (HSV-I) (HSV-II)	RG2	CL-2	ACL-2	NA – may not be moved to ACL1	HSV must be administered and animals must be housed under ACL-2 containment.	Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach). Minimum 20 minute contact time	
Vesicular stomatitis virus (VSV)	RG2	CL-2	ACL-2	NA – may not be moved to ACL1	VSV vectors must be administered to animals and animals must be housed under ACL-2 containment.	Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach). Minimum 20 minute contact time	
Rabies Virus (RABV)	RG2	CL-2	ACL-2	NA – may not be moved to ACL1	RABV vectors must be administered to animals and animals must be housed under ACL-2 containment.	70% Ethanol, phenol, formalin, Lysol. 15 minute contact time.	

*Holding time that must be observed before animals can be downgraded to ABSL1 housing
** Autoclave initial cage change regardless of the hold period

APPENDIX B: CDM SPECIFIC REQUIREMENT

- 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 a new water bottle immediately after exposure.
- Do not cage change during “shedding” unless necessary – e.g. flood or sick animal. “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. Refer to Appendix A for shedding period for common viral vectors.
- 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 a cage with an **external 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/shedding.
- 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 countertop 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.