

Working with Retroviruses/Retroviral Vectors and Post Exposure Plan (PEP).

Background

The family of Retroviridae, consists of two subfamilies, and multiple genera within those subfamilies. Members of these genera are classified as simple or complex, depending on their integrative potential. Complex viruses include [Lentiviruses](#), which are included in a separate document. This document focuses on simple retroviruses, such as alpha-, beta-, gamma-, delta-, and epsilon retroviruses, which will be combined and referred to retroviruses for the remainder of this document. Retroviruses are composed of a nucleocapsid containing two copies of single-stranded, positive-sense RNA. Retroviruses have the ability to integrate DNA into the host genome of dividing cells, allowing for the viral DNA to be passed on to progeny cells after cell division. As a result, retroviral vector systems are useful tools to achieve genomic integration and stable gene expression of the gene of interest. While viral genomic integration is essential to obtain a stable expression of the gene of interest, it may potentially contribute to insertional mutagenesis. The primary safety concern is the oncogenic potential due to integration of the gene of interest into host DNA as it has been observed in human gene therapy.

Retroviral systems are typically designed from murine retroviruses and can be grouped into one of three classes; ecotropic (infecting only murine cells), amphotropic (can infect human cells) or pseudotyped (vector particles express glycoproteins and can infect human cells). Retroviral vectors pseudotyped with a murine envelope to allow infection of murine cells decreases risks as these viral vectors have decreased chances of infecting human cells. Other retroviral vectors can be pseudotyped using different envelope proteins, such as Vesicular Stomatitis Virus (VSV) G protein. This increases the tissue tropism as the VSV-G protein binds to a broad range of cell types and species. This also increases risk of infection following an accidental exposure as the virus will be able to infect any cell at the site of inoculation. Pseudotyping retrovirus also increases the stability of the viral particles to enable concentration of virus via ultracentrifugation. The tropism and transgene must be considered when determining containment level.

Modes of Transmission

Retroviruses and retroviral vectors may be transmitted by:

- Dermal inoculation via sharps “(needle-sticks), through scratches, cuts, abrasions, dermatitis or other lesions.
- Mucous membrane exposure of the eyes, nose, and mouth.
- Inhalation via aerosols depending on the use of equipment such as centrifuges or vortex mixers.

Laboratory Hazards

Risks include direct contact with skin and mucous membranes of the eye, nose and mouth, parenteral inoculation, and ingestion.

- High energy, aerosol-generating activities (centrifugation, sonication, high-pressure systems, vortexing, tube cap popping)
- Handling of sharps (needles, scalpels, microtome blades, broken glass, etc.)
- Splash/droplet-generating activities (shaking incubators, liquid culturing, mechanical pipetting)
- Equipment contamination
- Exposed skin/uncovered wounds/broken or chapped skin

Signs and Symptoms

While replication defective retroviral vectors do not typically cause disease in humans, they still have oncogenic potential or expression of unintended transgenes depending on the insert.

Prophylaxis

Post-exposure prophylaxis for occupational exposure to retroviral vectors may include the use of antiretroviral drugs to block reverse transcription and integration of a transgene into an individual's genome. Post-exposure prophylaxis is most effective when given as soon as possible within the first 72 hours after an exposure. If an exposure occurs, seek immediate medical evaluation.

Biosafety Requirements and Procedures

Physical Containment

Retroviruses and retroviral vectors may be worked with at varying biosafety levels, depending on the nature of the inserted genes, tropism, and their replication competence. Generally, retroviruses are classified as a Risk Group 2 (RG2) agent.

Consequently, retroviruses and retroviral vectors are typically worked with at Biosafety Level 2 (BSL-2), requiring BSL-2 practices and procedures for all work with the virus or vector and Animal Biosafety Level 2 (ABSL-2) for all animal manipulation as well as animal housing. The Institutional Biosafety Committee (IBC) may set higher containment practices and procedures (BSL-2+), depending on a risk assessment of the nature of the work, the vector's tropism, replication competency, and the gene insert(s).

Personal Protective Equipment (PPE)

The following personal protective equipment must be worn when working with retroviral vectors:

- Gloves (double gloves for BSL-2+)
- Lab coat (dedicated lab coat for BSL-2+)
- Eye protection
- Closed-toe shoes; long pants or equivalent
- Surgical masks and/or face shield are recommended anytime there is a risk of aerosol/splash/spray of retroviral particles to the face outside the BSC. In some cases, an N95 respirator (annual fit testing required) might be required.

Spills

Spill Inside Biosafety Cabinet (BSC) or Containment

1. Keep the biosafety cabinet on and do not lower the sash.
2. Wear appropriate PPE (laboratory coat, gloves, safety glasses, etc.) and obtain or prepare fresh disinfectant solution for spill clean-up (10 % bleach solution or effective EPA-approved disinfectant).
3. Place paper towels or other absorbent material on top of the spill zone
4. Starting from the outermost edge of the spill and working in toward the center of the spill, pour disinfectant onto the absorbent material and allow sufficient contact time to inactivate any biological contaminants. Contact time is dependent on the pathogen/disinfectant combination but is typically in the range between 5 to 30 minutes. Avoid splashing and creation of aerosols.
5. After appropriate contact time, gather absorbent material and dispose waste into red biohazard bag. **Note, if using a large volume (> 1L) of disinfectant solution, consider placing the waste into a clear chemical waste bag and dispose of as chemical waste using the WASTE system.*
6. Disinfect the area again following steps 3-5 (if using bleach as a disinfectant, do a final wash of the area with 70% alcohol or water to prevent corrosion of the biosafety cabinet).
7. Remove gloves.
8. Wash hands.
9. Report the spill to the PI/Lab Manager/Supervisor.

Spill Outside Biosafety Cabinet (BSC) or Containment

1. Notify all personnel in the area and mark the area where the spill occurred to avoid the potential for cross contamination and unnecessary exposure to others in or near the work area.
2. Evacuate the immediate vicinity and close the door, if possible. Post signage to prevent anyone from entering the space.
3. Remove any contaminated clothing and wash exposed areas with mild soap and water for 15 minutes.
4. Report details and/or request assistance.
 - EH&S During business hours (951) 827 – 5228
 - UCPD Emergency 9-1-1
 - UCPD Non-Emergency Non-Business Hours (951) 827 – 5222
5. Wait 30 minutes to allow aerosols to settle or vent.
6. Wear appropriate PPE and obtain or prepare fresh disinfectant solution for spill clean-up (10 % bleach solution or EPA-Approved disinfectant).
7. Place paper towels or other absorbent material on top of the spill zone
8. Starting from the outermost edge of the spill and working in toward the center of the spill, pour disinfectant onto the absorbent material and allow sufficient contact time to destroy any biological contaminants. Contact time is dependent on the pathogen/disinfectant combination but is typically in the range between 5 to 30 minutes. Avoid splashing and creation of aerosols.
9. After appropriate contact time, gather absorbent material and dispose waste into red biohazard bag. **Note, if using a large volume (> 1L) of disinfectant solution, consider placing the waste into a clear chemical waste bag and dispose of as chemical waste using the WASTE system.*
10. Disinfect the area again following steps 7-9 (if using bleach as a disinfectant, do a final wash of the area with 70% alcohol or water to prevent corrosion of the biosafety cabinet).
11. Remove gloves.
12. Wash hands.
13. Report the spill to your PI/Lab Manager/Supervisor.

Decontamination — * Note - This is not to be performed for personnel exposure

The most effective disinfectant against retroviruses and retroviral vectors is a 1:10 sodium hypochlorite (bleach) solution that is made fresh daily.

- To make this solution, dilute 1 part bleach to 9 parts tap water.
- Ensure a 15 minute contact time.

- Use this disinfectant for treatment of reusable equipment, surfaces, and liquid waste (final volume 1% bleach).

Other effective liquid disinfectants are available. Check with EPA-approved lists or with EH&S Biosafety Officer.

Other inactivation methods include autoclaving for 1 hour at 121°C or 250°F (15 lbs psi of steam pressure). *Note that none of the autoclaves at UCR are certified for waste inactivation. All biohazardous waste should be placed in double dual-test compliant (ASTM D1709 and ASTM D1922) RED biohazardous waste bags and in the EH&S provided biohazardous waste collection bins for vendor pickup and disposal.*

Laboratory Practices

- No work with retroviruses or retroviral vectors is permitted on the open bench.
- A Certified Class II Biological Safety cabinets must be used for all manipulations including (but not limited to):
 - Pipetting
 - Harvesting infected cells for RNA isolation
 - Purification of virus or vectors
 - Infection of cell culture
 - Infection of animals
- Centrifugation must be done in closed containers with sealed rotors or sealed cups. Safety cups are to be loaded and unloaded inside the biosafety cabinet.
- All vacuum lines must be fitted with a HEPA filter.
- Vortexing must be done in the biosafety cabinet.
- Storage of retroviral stocks must be in leak-proof secondary containers (i.e. freezer boxes) in a -80° freezer clearly marked with a warning label to indicate that retrovirus is present.
- During active manipulation, minimize the creation of aerosols or splashes.
- All laboratory staff working with or supervising work with retroviruses or retroviral vectors must be made aware of the hazards associated with the work, required safety practices and procedures, and proper handling of the agent, as well as be current on required laboratory safety and biosafety trainings.
- Animal carcasses must be placed in biohazardous waste bags for waste disposal.
- Inoculation of animals must be performed in a BSC or other primary containment equipment.
- Cages must be labeled to identify inoculated animals and include the vector name, date of inoculation, and the biohazard symbol.

- Animal care and husbandry must be performed by laboratory staff for the first cage change and first 72 hours after inoculation. Following the first cage change after 72 hours, vivarium staff may take over standard husbandry duties.
- Cage changes within the first 72 hours and first cage change after inoculation must be treated as biohazardous.
 - All bedding and disposable cages must be disposed of as biohazardous waste.
 - Reusable cages must be decontaminated prior to washing.
- Signs and labels (universal biohazard symbol) must be placed to indicate each area where retroviruses or retroviral vectors are used or stored (including biosafety cabinets, incubators, refrigerators, laboratory entrance doors, etc.) The door signs should include the biosafety level, name of the agent, emergency contact information, PPE requirements, and the universal biohazard symbol. Contact EH&S for stickers/labels and sign templates.

Waste Disposal Procedures

- **Sharps Waste:** Place all sharps in red sharps container that is rigid, leak proof, and has the international biohazard symbol. Do not recap needles. Once $\frac{3}{4}$ full or at the fill line, close and secure the lid. Closed and secured sharps containers can be placed in the EH&S provided biohazardous waste collections bins for pickup and disposal. Additional sharps containers can be requested for free from EH&S.
- **Solid Waste:** Label a dual-test compliant red biohazard bag with PI name, building and room number before filling it. Bag should be placed in a hard-walled, leak-proof container with a tight fitting lid before adding waste. Within 7 days of first waste generation or once bag is $\frac{3}{4}$ full, close the bag and transfer to EH&S provided biohazardous waste collection bin.
- **Liquid Waste:** Prepare a compatible waste bottle with a tight fitting cap. Add enough undiluted bleach to the bottle to achieve 10% final concentration of bleach (1 part bleach: 9 part liquid waste). Cap tightly, invert, and gently mix contents to ensure bleach contacts all inner surfaces of the bottle. Let bottle sit for appropriate contact time (30 minutes) before pouring down laboratory sink drain followed by copious amounts of water to flush the pipes. If using another liquid disinfectant, create and affix WASTE label to bottle and dispose of as chemical waste following the WASTE process.

Immediate Response Following Exposure

- Eyes or Mucous Membrane Exposure from Splash or Aerosols - rinse a minimum of 15-minutes using eye wash and report to your supervisor immediately. Follow *Reporting Exposure Incidents* protocol.

- Skin Contamination - Wash affected areas with soap and water for 15-minutes and report the incident to your supervisor immediately. Follow *Reporting Exposure Incidents* protocol.
- Needlestick and/or Sharps Exposure – Wash and express wound with soap and water for 15 minutes. Immediately notify your supervisor. Follow *Reporting Exposure Incidents* protocol.

Reporting Exposure Incidents

Any exposure incident—such as contact of retroviral vectors with eyes, nose, mouth, skin contamination, needlestick and/or sharps exposure—must **be immediately reported** to:

- **Your PI or laboratory supervisor**
- **UCR Biosafety Officer (BSO) and EHSRM at (951) 827-5528.**
- **Occupational Health ehsochealth@ucr.edu**
- **You may contact the UCI Medical Center Infectious Disease Fellow on call at 714-456-6011 for immediate counseling and guidance. UCR maintains an agreement with the UCI Center for Occupational and Environmental Health (COEH) Clinic, which serves as our Occupational Health provider and reviews UCR's Animal Occupational Health Program.**

Undergraduate Student Employees report your injury to your supervisor (or go to [Employee Injuries](#)).

For life-threatening injuries, call 911 immediately.

For all other injury types, seek Medical Treatment at UCR's preferred Occupational Clinics. Visit the [Medical Treatment Facilities](#) webpage to learn more about where to seek medical treatment.

References

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[UC Irvine "SOP Working with Retroviral Vectors"](#)

[UC Riverside "Lentiviral Vectors Standard Operating Procedures"](#)

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University of California, Riverside
INFECTIOUS AGENT CARD



My job requires me to work with Retroviruses or Retroviral vectors and/or animals injected with them.

If the person with this card exhibits any of the symptoms listed on the back, immediately contact the UCI Medical Center Infectious Disease Fellow on call at (714) 456-6011. For immediate medical counseling on what to do right away, proceed to the nearest Emergency Department and present this card. For more information or to report an incident call: UC Riverside, Occupational Health, at (951) 827-5528.

Retroviruses and retroviral vectors are transmitted dermally via sharps (needlesticks), absorption through exposed scratches or abrasions on skin, or mucous membrane exposure, inhalation via aerosols.

INCUBATION PERIOD: Varies.

SYMPTOMS: Fever, headache, dehydration, weight loss, lethargy, nausea, vomiting, loss of appetite.

TREATMENT: Post-exposure prophylaxis for occupational exposure to retroviral vectors may include the use of antiretroviral drugs.

Acknowledgement of Working with Retroviral Vectors

By signing below, I confirm that I have reviewed and understood the requirements for working with retroviral vectors. I agree to comply with all outlined responsibilities, including:

- Following safe laboratory practices and use of appropriate PPE
- Applying proper first aid and decontamination procedures in the event of an exposure
- Promptly reporting any exposures, incidents, or safety concerns to my supervisor, Biosafety Officer, and Occupational Health

Name (Print)	Identification*	Signature	Date	Supervisor / Principal Investigator

*Identification: Provide your UCR Student ID, Employee ID, UCR NetID, UCR Email, or Date of Birth.