

Working with Lentiviral Vectors and Post Exposure Plan (PEP).

Background

Lentiviral vectors are based on the Human Immunodeficiency Virus (HIV) which is the virus responsible for the development of Acquired Immunodeficiency Syndrome (AIDS). Lentiviruses are a subclass of retroviruses which can infect both proliferating and non-proliferating cells. Lentiviral vectors have been modified to provide a safer version of the HIV virus in which the viral replication genes have been removed. During infection, there is a possibility that the lentivirus may convert to a replication competent state. Although this scenario is highly unlikely, monitoring for such a possibility is encouraged, since such a conversion could compromise laboratory safety.

The major risks to be considered for research with HIV-1 based lentivirus vectors are the potential for generation of replication-competent lentivirus (RCL), and the potential for oncogenesis via random chromosomal integration. The nature of the transgene must also be considered in assessing risk. These risks can be mitigated by the nature of the vector system (and its safety features) or exacerbated by the nature of the transgene insert encoded by the vector (e.g. expression of known oncogene with a constitutive strong promoter may require heightened safety precautions)

The potential for generation of RCL from HIV-1 based lentivirus vectors depends upon several parameters, the most important of which are the number of recombination events necessary to reassemble a replication competent virus genome and the number of essential genes that have been deleted from the vector/packaging system. On this basis, later generation lentivirus vector systems are likely to provide a greater margin of personal and public safety than earlier vectors, because they use a heterologous coat protein (e.g., VSV-G) in place of the native HIV-1 envelope protein, thus reducing the risk of RCL generation. (It should be noted, however, that pseudotyping with coat proteins such as VSV-G may broaden the host cell and tissue tropism of lentivirus vectors, which will be considered in the overall safety assessment by the IBC). Later generation vector systems also separate vector and packaging functions onto three or four plasmids and they include additional safety features such as the deletion of Tat, which is essential for replication of wild-type HIV-1, and altered-3' LTR that renders the vector "self-activating" (SIN). In contrast, earlier vector systems (such as two-plasmid vector systems) may have a higher potential for generation of RCL.

Modes of Transmission

Lentiviruses may be transmitted by:

- Dermal 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, ingestion.

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

Signs and Symptoms

Lentiviruses may persist lifelong due to their ability to integrate into the host chromosome and ability to evade host immunity. Signs and symptoms of infection may include:

- Flu-like symptoms (i.e. fever, headache, dehydration, weight loss, lethargy)
- Gastrointestinal symptoms (i.e. loss of appetite, nausea, vomiting, diarrhea)
- Neurological symptoms (i.e. loss of sensation, ataxia)
- Lymphoreticular symptoms (i.e. enlarged internal organs or lymph nodes)
- Reproductive Health concerns (i.e. abortion, fetal abnormalities)

Incubation Period

1 to 6 months.

Prophylaxis

Post-exposure prophylaxis for occupational exposure with HIV-based viral vectors may include the use of antiretroviral drugs. If an exposure occurs, seek immediate medical evaluation.

Biosafety Requirements and Procedures

Physical Containment

All work with Lentiviral vectors must be conducted utilizing a Biological Safety Level 2 (BSL2) or Biological Safety Level 2+ (BSL-2+) practices and procedures. This includes but is not limited to a room suitable for tissue culture and equipped with a certified Class II Biosafety Cabinet (BSC)

Personal Protective Equipment (PPE)

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

- Gloves (double gloves for BSL2+)
- Lab coat (dedicated lab coat for BSL2+)
- Surgical masks and eye protection or face field are recommended anytime there is a risk of aerosol/splash/spray of lentiviral particles to the face outside the BSC. In some cases, an N95 respirator (annual fit testing required) might be required.

Engineering Controls

The following safety equipment must be used when working with Lentiviral vectors:

- Certified Class II Biological Safety cabinets.
- Sealed centrifuge rotors and/or safety cups, and
- Vacuum lines equipped with an in-line HEPA filter as well as a primary and secondary vacuum flask containing a 10% bleach solution.

Spill Kit

The lab must have a spill kit or the components of such readily accessible in the event of a spill. This comprises an easy-to-read outline of the spill response SOP, gloves, masks, goggles, clean lab gown or lab coat, paper towels to absorb contaminated liquids, disinfectants, tongs or forceps to pick up broken glass and a red biohazard bag.

Decontamination/Clean-up Procedures

All materials that have come into contact with Lentiviral vectors should be disinfected using a 1:10 bleach solution before disposal. Additionally, all work surfaces must be disinfected with a 1:10 solution of bleach once work is completed and at the end of the workday. (Note: a 15-minute contact time is required for decontamination).

Special Handling Procedures

- Cells exposed to Lentiviral vectors may not be removed from the laboratory for experimental purposes unless inactivated by approved procedures.

- If you need to aerate cultures, it must be done slowly and in a manner that minimizes the potential for aerosol creation. This action must be carried out in a class II biological safety cabinet.
- When pouring and pipetting samples, it must be done gently and slowly and must be carried out in a class II biological safety cabinet.
- Extra precautions must be taken when using sharps. Appropriate substitutes for sharp items must be used whenever they are available. No sharps (including needles and Pasteur pipettes) may be used for working with Lentivirus-infected cell cultures nor when harvesting virus pellets. Use plastic aspiration pipettes instead of glass Pasteur pipettes.
- For Aspiration, use a plastic vacuum flask with a second vacuum flask connected to it as a backup, with non-collapsible tubing capable of withstanding disinfection. To the second vacuum flask attach a hydrophobic and a HEPA filter (or combination filter) to ensure that nothing is sucked into the house vacuum system. These 3 items must be attached in series from the vacuum source in the hood or a vacuum pump.

Waste Disposal Procedures

- Non-Sharp Waste—All cultures, stocks, and cell culture materials must be disinfected and autoclaved prior to being disposed of into a double red-bag lined biohazard box.
- Sharps Waste—All needles, syringes, razors, scalpels, Pasteur pipettes and pipette tips must be disposed of in an approved, puncture resistant sharps container. Sharps container must not be filled more than 2/3 of their capacity.

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 affected areas 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 lentiviral 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 ehsocchealth@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

Biological Safety Principles and Practices, 3rd edition, 2000 ASM Press. Edited by Diane O. Fleming, Ph.D., and Debra Hunt, Dr. P.H.

[Biosafety in Microbiology and Biomedical Laboratories](#), 6th edition, December 2024. Centers for Disease Control.

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[Guidelines for Research Involving Recombinant DNA Molecules](#), April 2024. National Institutes of Health.

[National Institutes of Health \(NIH\). Biosafety Considerations for Research with Lentiviral Vectors](#).

[Risks Associated with Lentiviral Vector Exposures and Prevention Strategies](#). December 2016. *Journal of Occupational and Environmental Medicine*, 58(12), 1159-1166. Schlimgen, R., Howard, J., Wooley, D., Thompson, M., Baden, L.R., Yang, O.O., Christiani, D.C., Mostoslavsky, G., Diamond, D.V., Gilman Duane, E., Byers, K., Winters, T., Gelfand, J.A., Fujimoto, G., Hudson, T.W., Vyas, J.M.

Appendix A: Post Exposure Print & Go Sheet

What are print and go sheets?

Following an occupational exposure to the agent identified above, this information sheet identifies the immediate “first aid” actions that should be taken. A medical evaluation should be sought immediately following the exposure. The guidance sheet provides information that medical personnel can reference but does not provide individualized medical care or treatment. This sheet should be printed and taken to the medical provider.

Organism and Exposure

Lentiviruses are a genus of the retrovirus family and include; HIV, SIV, SHIV and FIV among others. The wild type HIV backbone has been modified for research and therapeutic applications such that it can be used as a carrier vehicle; called a lentiviral vector, to efficiently introduce genetic material (transgenes) into both dividing and non-dividing target cell genomes. 3rd and 4th generation lentiviral vectors typically used today are considered to be replication incompetent. Replacement of the wild HIV envelope gene with vesicular stomatitis virus glycoprotein (VSV-G) gene broaden the cell types that can be infected (wild HIV targets CD4 cells) and the modes of transmission beyond percutaneous and mucocutaneous modes to also include potential aerosol risks. Hazards of a lentiviral vector include the effects of the expressed transgene such as an oncogene, or toxin being introduced into the target cell by the vector. The onetime introduction of a gene can introduce potential problems which are hard to gauge and may be long term. In addition, for replication incompetent lentiviral vectors, while the virus does not replicate, the transgene is integrated into the host genome. The transgene may also insert in a genetically sensitive area and induce mutational changes. This is called insertional risk.

Post-exposure Medical Surveillance:

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.

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.

Give this sheet to the physician so they understand that you may have been exposed to a lentiviral vector, and this is a medical emergency.

1. **Verify that first aid was performed** – Ensure skin was washed with soap and water for 15 minutes and mucus membranes or eyes with plain water for 15 minutes. Confirm that the area of injury is not squeezed and chemicals like bleach are not used as they are not known to be beneficial and may break down the barrier function of the skin.
2. **Document and understand the exposure** – This means the type of lentiviral vector (e.g. HIV backbone), generation, replication incompetent or competent, transgenes of concern like oncogenes, good gene knockdown or knockout genes, or toxins carried by the vector. Confirm what type of animal, cells or tissues are being used as these may present separate hazards; including ordinary bloodborne pathogens (human cells or tissues), zoonoses, chemicals or drug exposures. Note that not all cells and tissues are screened for bloodborne pathogens prior to use in research, and macaque cells and tissues may harbor macacine herpes virus 1 (herpes B virus). Confirm if exposure was mucocutaneous, percutaneous, or aerosol and how large the exposure was as well as when it occurred along with viral vector titer. Determine the nature of the research, contacting the principal investigator (PI) if needed with the exposed individuals' permission, to fully understand the potential hazards.

Testing and Follow-up

Testing for lentiviral vector exposure is generally not helpful. HIV testing may be worthwhile as if someone has undiagnosed HIV, the PEP regimen could produce HIV resistance, and there is some potential risk for wild HIV recombination with the lentiviral vector. The post exposure visit documents the exposure and so the extent of documentation if something arises down the road may be quite important.

Baseline and follow-up labs are indicated for those started on medications and depend upon medications but generally include CBC and diff, BUN/creatinine and AST + ALT.

Medication

Replication incompetent lentiviral vector exposures – There are no studies of the benefits or risks of post exposure prophylaxis for insertional risks. There are no national, published guidelines or consensus on this although a group of national experts have just been funded to develop protocols in this area. Based on guidance from the UCR Occupational Health reviewing physician and established best practices, including Columbia University [“Print-and-Go” Lentiviral Vector Post-Exposure Guidance](#) it is **highly recommended that an exposure evaluation be conducted as soon as possible—ideally within two hours, and no later than 12 to 24 hours post-exposure**—to determine the need for post-exposure prophylaxis and minimize potential insertional risks, particularly when working with lentiviral vectors containing hazardous transgenes. After 72 hours there is no likely benefit. Exposure to cells or animal tissues that

have been transduced with a lentiviral vector presents minimal risk and there is no likely benefit to post exposure prophylaxis, especially if the transduction occurred more than 72 hours ago.

Recommended regimen—2 drugs; Dolutegravir (Tivicay) 50 mg BID with or without Tenofovir (Viread) 300 mg once daily for 7 days or Dolutegravir + Emtricitabine for 7 days can be used as alternative drugs (some institutions use 7 days) may be used in the event of a worrisome transgene. This is off label use. Protease inhibitors (like Kaletra) have no effect on transduction or integration of the lentiviral vector and therefore are not used for insertional hazards.

Replication competent lentiviral vector—should be treated just like a wild type HIV BBP exposure so use the normal HIV exposure protocol for this; such as Dolutegravir and Truvada for 28 days: Be sure to know the drug side effect profiles and any drug-drug interactions, following labs which typically include BMP and LFT at baseline and follow up while on treatment. A common example is the issue of renal toxicity and renal insufficiency dosing with Tenofovir in Truvada or as a single agent in Viread.

Next Steps

If evaluated at the ER, follow up with respective campus provider as soon as possible, notify supervisor of incident, complete incident report. By law, any exposures to recombinant DNA (which includes lentiviral vector) must be reported to the NIH, by notifying EHSRM and your institutional IBC. **UCR Biosafety Officer (BSO)** or **Occupational Health** ehsocchealth@ucr.edu **(951) 827-5528**

References:

This document is adapted from [Risks Associated with Lentiviral Vector Exposures and Prevention Strategies](#). December 2016. Journal of Occupational and Environmental Medicine, 58(12), 1159-1166. Schlimgen, R., Howard, J., Wooley, D., Thompson, M., Baden, L.R., Yang, O.O., Christiani, D.C., Mostoslavsky, G., Diamond, D.V., Gilman Duane, E., Byers, K., Winters, T., Gelfand, J.A., Fujimoto, G., Hudson, T.W., Vyas, J.M.

Public Health Agency of Canada. Lentiviral Vectors Guideline 2019. Ottawa: Government of Canada; 2019. Available from: https://www.canada.ca/content/dam/phac-aspc/documents/services/canadian-biosafety-standards-guidelines/guidance/lentiviral-vectors/Lentiviral_Vectors_Guideline_2019.pdf

Columbia University Environmental Health and Safety. (n.d.). *Lentiviral Vector Post-Exposure Prophylaxis: Print-and-Go Guidance*. Columbia University Research Safety. Retrieved from <https://research.columbia.edu/sites/default/files/content/EHS/Homepage/LentivirusPostExposureProphylaxisPrintAndGo.pdf>



University of California, Riverside
INFECTIOUS AGENT CARD



My job requires me to work with the Lentiviral vectors and animals injected with Lentiviral vectors.

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.

Lentiviruses are transmitted by dermal via sharps (needlesticks), absorption through exposed scratches or abrasions on skin, or mucous membrane exposure, inhalation via aerosols.

INCUBATION PERIOD: 1 to 6 months.

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

TREATMENT: Post-exposure prophylaxis for occupational exposure with HIV-based viral vectors may include the use of antiretroviral drugs.

Acknowledgement of Working with *Lentiviral* vectors

By signing below, I confirm that I have reviewed and understood the requirements for working with Lentiviral 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.