Lentivirus and Lentiviral Vectors

Risk Group: 3

Health Hazards

Lentiviruses are a subset of retroviruses that have the ability to integrate into host chromosomes and to infect non-dividing cells and include human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) which can infect humans. Another commonly used lentivirus that is infectious to animals, but not humans, is feline immunodeficiency virus (FIV). Lentiviral vectors consist of recombinant or synthetic nucleic acid sequences and HIV or other lentivirus-based viral packaging and regulatory sequences flanked by either wild-type or chimeric long terminal repeat (LTR) regions.

Use of these vector systems is particularly desirable because of their ability to integrate transgenes into dividing, as well as non-dividing cells. However, there are risks associated with working with lentiviral vectors and these must be carefully evaluated.

The two major risks are: 1) the potential generation of replication competent lentivirus (RCL); and 2) the potential for oncogenesis. These risks are largely based upon the vector system used and the transgene insert encoded by the vector. Therefore, it is imperative that prior to utilizing a lentiviral vector system, a risk assessment must be reviewed and documented. Also, because construction and/or use of lentiviral vectors falls under the definition of r/sNA research as **outlined in the NIH Guidelines, possession must be reported in the workplace's biological agent inventory and experiments must be submitted for review and approval by the site IBC prior to initiation.**

Modes of Transmission

Lentivirus is transmissible through injection, ingestion, exposure to broken skin or contact with mucous membranes of the eyes, nose and mouth. **Immunocompromised individuals should not work with lentivirus**.

Administrative Controls

- Access to the laboratory shall be restricted, doors must remain closed during experimentation.
- Signs and labels (universal biohazard symbol) must be placed to indicate each area where

lentiviruses are used or stored (including biosafety cabinets, incubator, refrigerators, laboratory entrance doors, etc.) The signs should include the name of the agent, emergency contact information and a biohazard sticker. ALL laboratory personnel must be advised of the hazards of the agent

- All laboratory personnel must be trained in the proper handling, use and disposal prior to working with agent
- All laboratory personnel must remove lab coats, discard gloves in the proper biowaste container and wash hands before exiting the lab
- Standard Operating Procedures (SOP's) for the planned procedures must be written and shall be present in the laboratory at all times

Engineering Controls

- **Biosafety Cabinet:** All Lentiviral vector work must be performed in the BSL-2 laboratory and require a **Certified Class II BSC**. If the blower on the BSC is not left on continuously, it should be turned on and run for 5 min. to allow several complete exchanges of air before work can begin. When double-gloving (optional), remove the outer pair of gloves and deposit in a solid waste bag before removing hands from the BSC. At the end of the work session, all items to be removed from the BSC must be decontaminated. The surface of the BSC must be wiped down with 10% bleach and followed up with 70% EtOH.

- **NO** open-bench work!

- **Signage**: During work with viral particles, a warning sign must be posted on the door alerting personnel of the presence of lentiviral particles.

- **Biohazard Sharps Containers** shall be available to dispose of sharps waste, including broken glass, needles, blades, etc.

- **Centrifugation** must be performed in closed containers and using **sealed rotors or safety cups** to minimize the risk of aerosol generation. **Samples must be placed into and/or removed from cups within a BSC.**

- **Vacuum:** all vacuum lines must be **fitted with a HEPA filter** and a **vacuum flask**, containing **10% bleach** in a volume sufficient to provide the recommended final concentration for that disinfectant when the flask is near full. At the end of the work session, aspirate a small volume of concentrated disinfectant through the vacuum tubing, into the vacuum flask. The vacuum flask must sit for a **minimum time of 30 minutes** prior to drain disposal.

- **Vortexing:** must be done in BSC.

- **Pipetting:** Aerosol resistant (filtered) tips must be used when pipetting.

- **Sharps (ONE-TIME USE):** All sharps should be immediately disposed of in to a sharps container (located within the BSC). Needles must never be recapped.

- Storage of **lentiviral** stocks must be in leak-proof secondary containers (i.e. freezer boxes) in a -80^0 freezer clearly marked with a warning label to indicate that lentivirus is present.

Personal Protective Equipment (PPE

The following PPE must be worn at ALL times when handling **Lentiviral vectors**.

X Gloves **X** Lab Coat **X** Safety Goggles/Glasses Shoe cover Gown
N95 Respirator
X Surgical Mask (recommended any time there is a risk of a splash of lentiviral particles to the face outside the BSC.)
Face Shield Other:

Disinfection

Lentiviral particles can be inactivated with a number of reagents, including (final concentrations) 10% bleach, 5% Amphyl (phenolic),

- **Disinfectants:** 10% Sodium Hypochlorite (1:10 dilution 5.25% household bleach, such as Clorox)
- **Contact time:** 30 minutes before cleanup

Disposal

Liquid waste must be aspirated into a vacuum flask containing 1/10 volume concentrated bleach. A common practice is to anchor the end of the vacuum tubing to the outside of the sash or frame of the Biosafety Cabinet. At the end of the work session, aspirate 25-50 ml of concentrated bleach through the vacuum tubing, into the vacuum flask. The vacuum flask must have a final concentration of at least 10% bleach, for a minimum time of 30 minutes prior to drain disposal. Liquid waste that is not aspirated must be treated with bleach, to a final concentration of at least 10% in the hood, allowing a minimum time of 30 minutes to inactivate virus or autoclaved. A simple 500 ml bottle with 100 ml concentrated bleach may be suitable to collect non-aspirated liquid waste.

Solid biohazardous waste, Everything that contacts virus-containing solutions or vessels must be decontaminated or contained before exiting the biosafety cabinet. Solid waste can

be collected in a biohazard bag or sharps container inside the Biosafety Cabinet. At the end of the work session, the biohazard bag will be closed, sprayed with 70% EtOH, and deposited into a biohazardous waste container.

Sharps waste, such as broken glass, pasteur pipets, razor blades, and needles, are disposed of into an approved biohazard sharps containers, autoclaved for 60 minutes, placed in a box-bag unit for pickup by EHS. NEVER RECAP NEEDLES

Practices for Animal Injections

Lentiviral vectors cannot replicate (even in wild-type form) in rodents. However, at the time of transfer of lentiviral vectors and/or lentiviral infected cells into the animals (e.g., by injection), the animals may still have infectious virus on their wound or body secretions that could be transmitted to laboratory personnel. IN addition, animals reconstituted with human tissues are theoretically able to support the replication of RCL. Unless otherwise specifically approved by the IBC, the use of lentiviral vectors for gene delivery in rodents, which do not hold human tissues, requires the following:

1. Use of third generation (or higher), 3 and 4-plasmid lentiviral vector systems in rodents with or without human cells present will be housed at ABSL-2 for the duration of the study. possible. Work with animals will require added precautions as determined by the Institutional Animal Care and Use Committee (IACUC).
2. The delivery of viral vector must be performed in Class II BSC under ABSL-2 conditions. Animals should then be housed in filter-pop cages.
3. There will be specific signage/labeling on each ABSL-2 cage stating, “Biohazard Lentiviral Vector,” and these changes will not be allowed out of the ABSL-2 containment space. Outer entry/exit doors must have signage displaying biohazard pathogen presence.
4. ABSL-2 cages and bedding must be decontaminated before they are cleaned and washed.

Additional precautions may be required as determined by the Institutional Care and Use Committee.

Spill and Exposure Procedures

See pages 5-7of the laboratory specific biosafety manual

Medical Surveillance

All laboratory personnel must be advised by the PI on the health hazards of the Lentivirus prior to starting work within the laboratory. In the event of an exposure or potential exposure, personnel

must seek medical attention. The medical treatment provider may require continued self-monitoring of symptoms or further testing, such as serology.

References

1. Biosafety in Microbiological and Biomedical Laboratories. Washington, D.C. Centers for Disease Control and Prevention and National Institutes of Health.
2. NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules. Bethesda. The National Institutes of Health Office of Biotechnology Activities.
3. Biosafety Considerations for Research with Lentiviral Vectors: Recombinant DNA Advisory Committee (RAC) Guidance Document. The National Institutes of Health Office of Biotechnology Activities.

Agent: Lentivirus and Lentiviral Vectors Training Record

I have reviewed and understand the risks associated working with Lentivirus and Lentiviral Vectors I understand that my signature below indicates I agree to comply and work safely with the said agent.

Lab Personnel	Training Date	Signature