

Guidelines for Research Involving Viral Vectors: Retroviruses and Lentivirus Vectors

Retroviruses are enveloped, single-stranded RNA viruses capable of infecting dividing cells. Upon infection, the RNA genome is reverse transcribed and integrates as a DNA provirus into the chromosomal DNA of the infected cell.

Lentiviruses are a group of retroviruses that are capable of infecting non-dividing cells.

Retroviral vector characteristics include

A cloning capacity of ~7.5kb.

Titer production around 10^8 .

Long term (stable) expression.

Potential Health Hazards

Retroviruses can act as insertional mutagens, due to their ability to integrate into the host's DNA. Transcriptional activation of host genes adjacent to the site of integration may result, a process dependent on the enhancer/promoter within the viral long terminal repeat (LTR). Replication defective retroviruses can recombine with endogenous retro-elements, thus reestablishing or enhancing the pathogenic potential of the virus undergoing recombination. While murine retroviruses are inactivated by human complement and are not capable of causing human disease, lentiviruses are not inactivated by human complement and can cause disease.

Modes of Transmission

Retroviral transmission can occur through non-intact skin or mucous membrane exposure, accidental parenteral inoculation, or ingestion. The hazard of aerosol exposure is unknown. HIV in particular can also be transmitted from person to person through direct exposure to infected body fluids (blood, semen).

Laboratory Acquired Infections

Occupational exposure to HIV has been documented in 57 cases of HIV seroconversion among health care workers in the United States (as of the last report in December of 2002); among those 57 cases, 26 developed AIDS. Five laboratory acquired infections with HIV have been

reported as a result of splashing of infected materials, in apparent skin exposure and puncture wounds.

While needle sticks can transmit the virus, less than 1% of HIV contaminated needle sticks have resulted in infection.

Host Range

The host range is dependent upon the viral envelope glycoproteins and structural proteins involved in integration. Possible hosts include human, murine, feline, bovine, and avian.

Survival

Survival in the general environment is poor. Drying in the environment can cause 90-99% reduction in HIV concentration within several hours.

Laboratory Practices

The appropriate biosafety level (BSL) practices and facilities will depend on the host range (envelope glycoproteins) and insert characteristics of the recombinant virus. A majority of the retroviral vectors are based on the murine leukemia viruses (MLV) which is a risk group 1 agent; however, the use of viral-based vectors for gene delivery to mammalian cells has been assigned BSL2 containment by the Institutional Biosafety Committee (IBC) unless justification for a lower classification is proposed by the PI and approved by the IBC.

Biosafety level 2 practices and facilities must be used for activities involving retroviruses and retroviral vectors (for HIV, see below).

- Biohazard signs and labels must be displayed in areas and on equipment where viruses are used and stored. This includes, but is not limited to, laboratory entrance doors, biological safety cabinets, incubators, refrigerators, and freezers.
- Use a biological safety cabinet (BSC) (a.k.a. tissue culture hood) for manipulations that can generate aerosols, such as pipetting, harvesting, infecting cells, filling tubes/containers, and opening sealed centrifuge canisters. If a procedure cannot be done in a BSC and only on an open bench, use a plastic shield to prevent exposure through inhalation or splashing.
- Use aerosol containment devices when centrifuging. These include sealed canisters that fit in the centrifuge bucket, covers for the centrifuge bucket, heat sealed tubes, or sealed centrifuge rotors. Rotors should be removed and opened inside a BSC. Centrifuge tubes should be filled and opened in a BSC.
- Vacuum lines must be protected with liquid disinfectant traps and/or micron filters.

Biosafety Level 2 with Biosafety level 3 practices and containment equipment must be used for activities involving research-laboratory-scale quantities of HIV, manipulation of HIV preparations and activities that may produce aerosols.

- All work must be done in a biological safety cabinet,

- Lab doors must be closed and remain closed when work is in progress. Access is restricted to those whose presence is required while work is in progress.
- Autoclave waste items as soon as possible and before the end of the day.
- Use only disposable plastic flasks, tubes, plates, etc. for culture materials.
- Pay strict attention to sharps safety and the use of safety devices.
- Standard operation procedures (SOPs) must be developed and include biosafety precautions, an emergency plan, spill plan, etc.

Biosafety level 3 practices and facilities must be used when preparing or manipulating concentrated quantities of HIV.

- Directional airflow is provided, drawing air into the laboratory from “clean” areas and toward “contaminated” areas.
- A biosafety manual is developed specific to the laboratory and biosafety precautions are incorporated into standard operating procedures.
- Respiratory protection may be required.
- Policies and procedures are developed such that only persons who have been advised of the potential biohazards, who meet any specific entry requirements, and who comply with all entry and exit procedures may enter the lab.

Personal Protective Equipment

Personnel protective equipment (PPE) includes, but is not limited to-

- Disposable gloves (nitrile, latex, etc.)
- Lab coat when working in laboratory. Remove when leaving.
- Goggles for splash protection.
- Closed toe shoes.

Precautions When Using Animals

- Inoculation of BSL-2 biohazardous materials (including, but not limited to: viral vectors and human tumor cell lines) must be performed within a Class II BSC*.
- Tissue harvest (including blood collection) must be performed in the necropsy suite or in a Class II BSC.
- ABSL-2 signage must be placed on the animal room door when BSL-2 agents are in use. See Department of Comparative Medicine for your room assignment and signage.
- Depending on hazard assessment as performed by the IBC, IACUC, and Attending Veterinarian, cages may be considered biohazardous. Please meet with Comparative Medicine prior to initiation of the animal experiments to discuss handling of soiled cages and other waste materials.
- All cages containing animals inoculated with biohazardous agents must be marked with:
 - The agent
 - The PI
 - The date of administration
 - Any special handling requirements of soiled bedding/cages.
- ABSL-2 carcasses are considered biohazardous and are incinerated.

*Deviation from using a Class II BSC must be approved by the IBC and/or IACUC Committee

Animal use requests are made to the Institutional Animal Care and Use Committee (IACUC).

A complete copy of USA's Animal Biosafety (ABSL-2) Guidelines can be found at:

<https://southalabama.edu/departments/research/compliance/animalcare/animal.biosafety.guidelines.pdf>

Recombinant Retroviral and Lentiviral Research

Protocols involving recombinant retroviral vectors must be approved by the Institutional Biosafety Committee (IBC)

Employee Exposure

Eye exposure - Rinse eyes with eyewash for at least 15 minutes.

Skin exposure - Cleanse the affected skin area immediately with surgical disinfectant soap, diluted Clorox (0.05%) or other approved disinfectant.

Report Incidents and Seek Treatment - Report actual or suspected exposure incidents to your supervisor immediately. An online incident report must be completed within 72 hours of the incident. This form can be found at: <https://iagasp2.southalabama.edu/incident/logon.aspx>
If possible, identify and secure the offending sample to contain its biohazardous content and to allow for testing if necessary.

Spills and Disposal Procedures

- If the spill area is large or in a common use area, mark the area so that others may avoid it.
- Using materials from you spill kit:
 - Don the appropriate PPE
 - Cover the spill with absorbent material
 - Pour disinfectant over the entire area and allow to stand for 30 minutes.
- Contact the PI and assess the magnitude of the spill and formulate further plans of action.
- Safely pick up any broken glass with tongs or sweep in to a dust pan.
- Place spill material in to an autoclave bag.
- Make sure that the area is cleaned and disinfected thoroughly.
- Soak contaminated clothes and shoes in a tray with approved disinfectant.
- Report all spills containing biohazardous or recombinant material to the Office of Research Compliance and Assurance at 460-6863.

Disinfectants

Disinfectants should be allowed a minimum of 20-30 minutes contact time. Use one of the following:

- Sodium hypochlorite (use 1-10% dilution of fresh bleach)

- 70% Ethanol
- 2% Glutaraldehyde, or Formaldehyde

Decontamination

Autoclave cultures for 30 minutes at 121°C or 250°F (15 lbs per square inch of steam pressure).
Disinfect work surfaces using an effective germicide (see above). This may be followed by an alcohol wipe to lessen the corrosive nature of the germicide.

Transport Requirements

Information and References

University of Iowa Environmental Health and Safety

<https://ehs.research.uiowa.edu/retroviruses-and-retroviral-lentiviral-vectors>

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