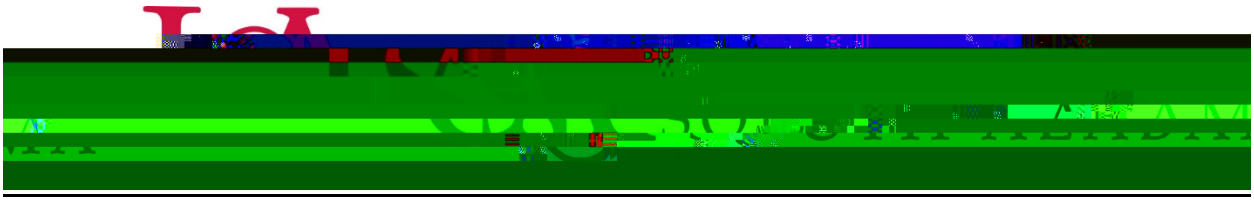

Guidelines for Research Involving Viral Vectors:
Retroviruses and Lentivirus Vectors

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 mutagen
or enhancing the pathogenic potential of the virus undergoing recombination.
etroviruses are inactivated by human complement and are not capable of
disease, lentiviruses are not inactivated by human complement and can cause

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.

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*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.10 (maMb5 (io)-2)10.2 (af)-

- 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/

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