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Lentiviral Vector - SAFETY FACT SHEET

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Overview

Lentiviruses form a genus within the Retroviridae family and encompass various subtypes such as bovine lentiviruses, equine lentiviruses, feline lentiviruses, and primate lentiviruses (e.g., Human immunodeficiency virus (HIV) types 1 – 3, Simian AIDS retrovirus SRV-1, Human T-cell lymphotropic virus type 4, and Simian immunodeficiency virus).

To ensure safety and adherence to guidelines, research involving lentiviral vectors must comply with the directives outlined in this guidance document and containment precautions prescribed by the Institutional Biosafety Committee (IBC).

Before handling lentiviral vectors or experiencing any potential exposure, researchers should have adequate knowledge about the properties of the **specific** lentiviral vectors used in their laboratories. Researchers should understand the transgene's function (e.g., oncogene, growth regulator or toxin), the number of plasmids required in vector generation, the host range, and the genes that have been removed.

In the event of exposure to a lentiviral vector within the laboratory setting, it is imperative to immediately report the incident and initiate appropriate measures as detailed in the OHP Guidelines. Rapid action is essential to ensure proper management and care.

Risk Review

The lentiviral vectors utilized in our laboratories are predominantly derived from HIV, the virus responsible for causing Acquired Immunodeficiency Syndrome (AIDS). These lentiviral vectors are highly efficient vehicles for *in vivo* gene delivery. Use of these vector systems are particularly vital to research by virtue of their ability to integrate transgenes into dividing, as well as, non-dividing cells. It is crucial to be aware of the major risks associated with lentivirus vectors:

1. Potential for the generation of replication-competent lentivirus (RCL).
2. Potential for oncogenesis.

The likelihood of generating replication-competent lentivirus from HIV-based lentivirus vectors is dependent on the following factors:

The number of recombination events required to assemble a replication-competent genome. Second-generation vector systems employ two helper plasmids to segregate the cis- and trans-factors, while third and fourth-generation systems involve three or more helper plasmids. The use of a vector with multiple plasmids reduces the risk of recombination events. Consequently, a third-generation system offers lower risk than a second-generation system.

The number of essential genes deleted from the vector system. Third-generation and subsequent vector systems either express the HIV regulatory Tat protein (which is crucial for the replication of wild-type HIV-1) under the control of a non-physiological ligand-regulated promoter, such as the tetracycline off system, or they exclude Tat.

Overall, later generation lentivirus systems present fewer risks due to the following reasons:

- The separation of vector and packaging genes onto four or more plasmids reduces the likelihood of recombination compared to a two-plasmid system.
- Essential genes are strictly regulated or eliminated (including Tat).

Insertional Mutagenesis

Insertional mutagenesis results from gene dysregulation at the site of the lentiviral vector integration within or near a coding region of the host genome.

While many of the lentiviral vectors do not replicate, the transgene may integrate into the host genome. The transgene may also insert in a genetically sensitive area and induce mutational changes.

Hazards of a lentiviral vector may include the effects of the expressed transgene such as a toxin, oncogene or inactivation of a tumor suppressor being introduced into the target cell by the vector. The one-time introduction of a gene can introduce potential problems, which are very difficult to assess.

The following tables summarize the biosafety concerns that should be considered when choosing a lentiviral vector system.

Biosafety Considerations and Risk Levels

Biosafety Considerations	Higher Risk	Lower Risk
Vector Design	• Vector packaging functions on two plasmids • Expression of viral genes	• Vector and packaging functions separated onto multiple plasmids • Deletion of viral genes
Transgene	• Oncogene	• Non-oncogene
Vector Generation	• Large scale	• Laboratory scale
Animal Hosts	• Permissive host • Animals engrafted with human cells	• Non-permissive host
Animal Manipulation	• Vector administration (e.g., use of sharps during injection	• Housing and husbandry (no use of sharps)

Source: NIH. Biosafety Considerations for Research with Lentiviral Vectors Recombinant DNA Advisory Committee (RAC) Guidance Document

Modes of Transmission

The most probable route of exposure for this work would be dermal via sharps (needle-sticks), absorption through exposed scratches or abrasions on skin, or mucous membrane exposure of the eyes, nose, and mouth. Another route would be inhalation via aerosols depending on the use of equipment such as centrifuges or vortex mixers.

Acknowledgement

As the Principal Investigator, it is your responsibility to ensure that all individuals listed in the IBC application are trained on accurate procedures for the safe handling of hazardous materials involved in this study. It is also your responsibility to ensure that your personnel complete all required training.

General Containment Considerations and Required Training

Biosafety Containment Requirements			
Viral Vector Type (replication incompetent vectors)	**Biosafety Level (BSL)**	**Animal Biosafety Level (ABSL)**	**Animal Biosafety Level After 72 hours (ABSL)**
Lentiviral Vector	BSL-2	ABSL-2	ABSL-1

- OH 201 Laboratory Safety - Required for all lab personnel working with hazardous chemicals or biological materials.
- OH 207 Biological Safety - Required for all lab employees working with biological hazards, e.g., infectious agents and recombinant DNA.
- OH 102 Hazard Communication (Site-Specific) - Taught by the laboratory's Principal Investigator or Supervisor. The training must be documented using the standardized HazCom Training Record - Labs Site-Specific (PDF) with checklist covering training topics that is available from EHS. Required for all lab employees working with or around hazardous chemicals.

Disinfection and Waste Disposal

It is necessary to disinfect all materials that have been exposed to Lentiviral vectors prior to disposal. To achieve this, it is recommended to use a fresh bleach solution, diluted 1:10. Furthermore, it is important to disinfect all work surfaces once the work is finished and at the end of each work day. All disinfected disposable materials that have come in contact with lentivirus must be disposed into the biohazardous waste stream.

Addition Materials/References

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

Viral Vector Biosafety in Laboratory Animal Research

ABSA Lentivirus Fact Sheet

University of Kentucky guidelines for research involving viral vectors

Lentivirus Use at Princeton University