INTRODUCTION

Lentiviral vector constructs have proven to be very productive in terms of transduction due to their ability to infect both replicating and non-replicating cells, including stem cells. Lentiviral vectors are becoming the vectors of choice for short-interfering RNA (siRNA) delivery. The increased use of lentiviral vector constructs in established and novel research applications makes it essential for laboratory workers to understand and protect themselves from related exposure hazards.

The purpose of this document is to provide principal investigators (PIs), laboratory technicians, and IBC members with information regarding risk assessment, proper work methods, containment levels, suitable engineering controls options, and personal protective equipment for development of research protocols that effectively reduce the risk of occupational exposure to lentivirus vector.

BACKGROUND

Third generation lentiviral vectors are usually created in a transient transfection system in which a cell line is transfected with multiple plasmid expression systems. These include:

1. The transfer vector plasmid (portions of the HIV provirus)
2. The packaging plasmid or construct
3. A plasmid with the heterologous envelop gene (env) of a different virus

The multiple plasmid components of the vector are put into a packaging cell which is then inserted into the HIV shell. These so-called split-configuration packaging cell lines require multiple recombination events to generate replication competent lentiviruses (RCLs).

A number of features are incorporated in the latest vector designs to enhance biosafety. These features include:

- Transgene: Non-oncogene; Vector and packaging components are distributed onto multiple plasmids that contain very little, if any, overlap or homology
- Deletion of viral genes (number of HIV genes is reduced to three (gag, pol and rev)
- Non-native viral env used in packaging system
- No expression of Tat (essential for lentiviral replication)
- Deletion in the 3’ LTR that results in “self-inactivation”
**RISK FACTORS**
The major risks to be considered for research with HIV-1 based lentivirus vectors are:

- Potential for generation of replication-competent lentivirus (RCL)
- Potential for oncogenesis

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.

**MODES OF TRANSMISSION**
Lentivirus may be transmitted by:

- Penetration of the skin via puncture or absorption (thought scratches, cuts, abrasions, dermatitis or other lesions)
- Mucous membrane exposure of the eyes, nose, and mouth

**GENERAL CRITERIA FOR RISK ASSESSMENT**
Decisions about containment should take into account a range of parameters/considerations including:

- The nature of the vector system and the potential for regeneration of replication competent virus from the vector components,
- The nature of the transgene insert (e.g., known oncogenes or genes with high oncogenic potential may merit special care)
- The vector titer and the total amount of vector,
- The inherent biological containment of the animal host, if relevant,
- Negative RCL testing

The potential for the generation of RCL from HIV-1 based lentivirus vectors depends upon several parameters:

1. The number of recombination events necessary to reassemble a replication competent virus genome
2. The number of essential genes that have been deleted from the vector/packaging system

**CONTAINMENT**
Generally, BSL-2 or BSL-2+ (enhanced BSL-2 containment including BSL-3 work practices and PPE) are often appropriate in the laboratory setting for higher generation lentivirus vectors with multiple safety features (four or more plasmids). Enhanced BSL-2 may include in addition to sharps safety, the use of PPE intended to reduce the potential for mucosal exposure to the vector (such as an N-95 respirator), especially if working with high volumes (>10L).
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
- Vacuum lines equipped with an in-line HEPA filter as well as a primary and secondary vacuum flash containing a 10% bleach solution.

PERSONAL PROTECTIVE EQUIPMENT
The following personal protective equipment MUST be worn when working with *Lentiviral* vectors:

- Gloves (consider double-gloving depending on the procedures being performed)
- Lab Coat
- Goggles
- Face shield

DECONTAMINATION PROCEDURES
All materials that have come into contact with lentiviral vectors should be disinfected using a 10% bleach solution before disposal. Additionally, all work surfaces must be disinfected with a 10% solution of bleach once work is completed and at the end of the work day. *(Note: A 10 minute contact time is required for decontamination)*

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 containers must not filled more than 2/3 of their capacity.

ANIMAL STUDIES
Some animals cannot support replication of infectious HIV-1; as a result, the potential for shedding of RCL is very low. It may be prudent to consider the biosafety issues associated with animal husbandry and housing after the initial injection separately from the inoculation itself, which may pose sharps hazards.

NIH recommends that the initial delivery of vector should be performed under BSL-2. It may be permissible to reduce the containment level at some point following vector delivery. An example is as follows: if there is no expectation of infection, the site of inoculation has been thoroughly cleansed, and the bedding changed, it may be acceptable to consider reducing containment from BSL-2 to BSL-1 within a few days (this time period is to be determined by the IBC and usually ranges between 1-7 days).
Stereostatic injections that require equipment that cannot fit in a biosafety cabinet, need to be taken into consideration during the risk assessment. BSL-2 + with stress on N-95 respirators may be suitable in this situation.

An important consideration is animals that have been grafted with human cells that are permissive for HIV-1 replication. These animals may require a higher level of containment.

*This document must be stored in the lab as a supplement to the lab-specific biosafety manual and it will be checked at the annual Environmental Health and Safety lab bio inspection. The vector-specific SOPs describing lentiviral work must be approved by IBC before lentivirus is used in the lab.

*The Principal Investigator is responsible for ensuring all lab personnel read this document and understand the information contained herein.

**REFERENCES**

   http://oba.od.nih.gov/rdna_rac/rac_guidance_lentivirus.html

2. *Biosafety in the Microbiological and Biomedical Laboratories, 5th ed.*  

*This page must be signed by lab personnel and will be checked during annual lab inspections by EH&S*

I am aware of the EVMS guidelines for working with lentivirus in the lab and of my lab-specific Standard Operating Procedures involving lentiviral work.

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