

Subject: Lentiviral Vector Use

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PROCEDURES FOR THE USE OF LENTIVIRAL VECTORS

PURPOSE

- To explain the risks associated with the use of lentiviral vectors in University of Ottawa laboratories and to ensure laboratories comply with the Public Health Agency of Canada's conditions for safe work with these vectors.

OVERVIEW

Lentiviral vector systems are widely used to transfer genes in cell culture systems and live animals. Many of these systems are derived from HIV-1, the risks of which include the generation of replication competent lentivirus (RCL) and the potential for oncogenesis through insertional mutagenesis. The use of later generation vectors in which 3 or 4 plasmids are used to produce the viral particles is encouraged where practical. A number of features are incorporated in the latest vector designs to enhance biosafety. These include:

- Minimalist design of the vector backbone (number of HIV genes is reduced to 3 (gag, pol, and rev) and a substantially deleted env gene)
- Vector and packaging components are distributed on four or more plasmids that contain very little homology (i.e. one of the packaging plasmids encodes the heterologous coat protein, VSV-G, which holds no homology to HIV-1 envelope protein)
- Deletion of 3' LTR that results in "self inactivation"
- Elimination of the TATA sequence essential for replication of wild type HIV-1

The result of these enhancements is a vector that has little capability of producing replication competent virus. Although these systems are much safer, the vectors can efficiently transduce human cells.

It is for this reason that a thorough risk assessment must be undertaken each time a new vector system is manipulated. Multiple factors must be taken into consideration when determining the risk associated with these vectors.

- Nature of the vector system and its potential for regeneration
- Nature of the transgene (i.e. oncogenes, toxin producing genes, antagonists of tumor suppressor genes etc.)
- Vector titer and volume (as these increase, so does the risk of exposure)

Biosafety Considerations and Risk Levels		
Biosafety Considerations	Higher Risk ←	Lower Risk
Vector Design	<ul style="list-style-type: none"> • Vector packaging functions on two plasmids • Expression of viral genes 	<ul style="list-style-type: none"> • Vector and packaging functions separated onto multiple plasmids • Deletion of viral genes
Transgene	<ul style="list-style-type: none"> • Oncogene 	<ul style="list-style-type: none"> • Non-oncogene
Vector Generation	<ul style="list-style-type: none"> • Large scale • Permissive host 	<ul style="list-style-type: none"> • Laboratory scale • Non-permissive host
Animal Hosts	<ul style="list-style-type: none"> • Animals engrafted with human cells 	
Animal Manipulation	<ul style="list-style-type: none"> • Vector administration (e.g., use of sharps during injection) 	<ul style="list-style-type: none"> • Housing and husbandry (no use of sharps)

Contact the Office of Risk Management, Biosafety Compliance Specialist for assistance.

Lentiviral vectors are classed as biohazard risk group 2 and must be handled in a containment level 2 laboratory using containment level 3 operational practices. In addition to the CL2 operational practices described in Public Health Agency of Canada's Laboratory Biosafety Guidelines, 3rd edition, the following containment level 3 operational practices must be followed:

1. Exterior street clothing such as coats, boots and gloves and all jewelry including watches must be removed before personnel enter the containment laboratory. Dedicated laboratory clothing such as a lab coat must be donned upon entering the laboratory and must be removed before leaving. Lab coats must be stored in the lab.
2. All personal items such as purses, bags, sweaters, coats and boots must not be brought inside the containment laboratory and therefore must be stored outside the lab in lockers or offices
3. Two pairs of gloves must be worn when manipulating the vector. Always treat your outer gloves as contaminated.
4. A solid front disposable gown must be worn over laboratory clothing when manipulating the vector. If a spill or splash occurs, autoclave and dispose of gown.
5. Limit the use of hypodermic syringes and needles and other sharps. Strongly consider the use of engineered sharps systems. If feasible, use plastic disposable transfer pipettes rather than glass Pasteur pipettes.
6. All manipulations of the vector must be conducted in a Biological Safety Cabinet. Only one person at a time working in the BSC.

7. Work in the BSC over an absorbent pad.
8. A small stand and autoclave bag must be placed in the BSC to collect waste (including disposable pipettes). Immediately upon completing work, seal it in a second autoclave bag and bring it to the central autoclave facility.
9. A tray containing freshly diluted 10% bleach solution must be placed in the BSC to collect reusable glass pipettes. Pipettes must be submerged for a minimum of 30 minutes.
10. A yellow sharps container must be placed inside the BSC for sharps disposal.
11. Centrifugation of infectious materials must be carried out in closed containers placed in sealed safety cups or rotors that are uploaded and unloaded in a BSC.
12. Inventory and storage locations of viral samples must be tracked.
13. Containment laboratory doors must be kept closed at all times and must be locked when unoccupied.
14. Lentiviral vectors must be stored inside the containment laboratory.

LENTIVIRAL VECTOR USE FOR IN VITRO STUDIES

1. VACU-GUARD Vacuum Protection Filter Device must be installed in the vacuum line to prevent aerosol contamination. (ex. Whatman Vacu-Guard, PTFE Membrane, 50mm diameter, Cat. #6722-5000)
2. When disposing of media from the vacuum flasks, a 10% volume of bleach must be added to the flasks and allowed to sit for 30 minutes (cover with parafilm), prior to disposal down the drain. If the flask sits overnight, it is recommended to leave the flask inside the fume hood.
3. In order to decontaminate vacuum lines, once the work is complete, concentrated bleach must be aspirated into the collector flask and let sit for 30 minutes contact time.
4. Designate an incubator for use with lentivirus. The dishes containing lentivirus should be put in a secondary container to move from the Biological Safety Cabinet to the incubator to reduce the risk of spills

LENTIVIRAL VECTOR USE FOR IN VIVO ANIMAL STUDIES

Animal Studies requiring Containment Level 1 (CL1)

Wild-type mice are not permissive for HIV-1. As a result, the potential for shedding of RCL from such animals is very low. To conduct studies on mice using lentiviral vectors the following procedure is recommended:

- The initial delivery of lentiviral vector should be performed under Containment Level 2 or under enhanced Containment Level 2 (See CL2+ requirements above). However, within 7 days of injection, if there is no expectation of infection, the site of inoculation has been thoroughly cleansed, and the bedding changed, the containment can be reduced from CL2 to CL1.
- Stereotactic injections that require equipment that cannot fit in a biosafety cabinet must be taken into consideration during the risk assessment. An N-95 respirator may be suitable in this situation. **N.B.** You must be properly fit-tested by a designated person prior to using an N-95 respirator.
- Mice are not permissive hosts for non-human lentiviral vectors such as Feline Immunodeficiency Virus replication. Containment Level 1 is acceptable for mouse housing and husbandry.

Animal Studies requiring Enhanced Containment Level 2 (CL2+)

Animal studies which would continue to require an enhanced Containment Level 2 (because of the potential for replication of HIV-1) include:

- Animals engrafted with permissive cells (e.g., human cells) or mice lines that are permissive for HIV-1 replication (e.g., SCID mouse with human immune system)
- Animal hosts that are permissive for HIV-1 replication
- Non-human lentiviral vectors such as FIV (feline immuno-deficiency virus) containing heterologous envelope proteins e.g., VSV-G (which could extend the tropism of the vector so that these vectors can transduce human cells)

FIRST AID

Modes of Transmission

- High Risk Exposures:
 - i. Skin puncture or injection
 - ii. Ingestion
 - iii. Contact with mucous membranes (eyes, nose, mouth)
 - iv. Contact with non-intact skin
- Low Risk/Potential Exposure:
 - i. Bite from a recently infected animal
 - ii. Percutaneous contact with body fluids from a recently infected animal
 - iii. Aerosols

First Aid

- Should a skin exposure occur, immediately go to the sink and thoroughly wash the area with soap and water.
- For a skin wound, immediately go to the sink and thoroughly wash the wound with soap and warm running water for 15 minutes. Gently work the blood toward the wound and pat dry.
- A splash to eye(s), nose or mouth requires immediately flush of the area with running water for at least 10 minutes.
- A splash affecting garments requires removal of the garments that have become soiled or contaminated, place in an autoclave bag and decontaminate before washing.
- Seek follow-up medical attention if required.
- Report the incident to your supervisor and complete a University of Ottawa Accident, Incident Report Form.

REFERENCES

Cornell University, Cornell University Institutional Biosafety Committee Guidance on the Use of Lentiviral-Based Vectors, 2008

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National Institutes of Health, Recombinant DNA Advisory Committee, Guidance on Biosafety Considerations for Research with Lentiviral Vectors (March 2006)

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