Working with Lentiviral Vectors

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Section 1. ADMINISTRATIVE OVERVIEW

A. PURPOSE-
The Purpose of this bulletin is to provide an overview of the hazards of working with specific lentiviral vectors/constructs and detail minimum operational practices for work with these biological agents in the research lab at the University of Manitoba (U of M).

This information is provided in the context of the Canadian Biosafety Standards (CBS) and the requirements to perform a Local Risk Assessment (LRA).

B. SCOPE-
   • The biological agent risk assessment and biosafety operational practices have been developed with the kind consultation of Dr. Sam Kung, Associate Professor, Department of Immunology and Dr. Mojgan Rastegar, Associate Professor, Department of Biochemistry and Medical Genetic.
   • The operational practices reflect work with recombinant Human Immunodeficiency Virus (HIV-1) based vectors assembled from three (or four) plasmids using a packaging cell line. They use a VSV-g pseudotyped virus envelope that has been engineered to be replication incompetent (only one round of infection should occur). Detailed information is provided in Section 2 Pathogen Risk Assessment.
• This SOP is for in vitro work in a research lab. A safe work practice for in vivo work is located on the Animal Care Occupational Health website and includes the specific Schedule10 for Lentiviral Vectors in Animals.

C. RESPONSIBILITY-
It is the responsibility of the Principal Investigator, Laboratory Supervisor, and responsible owners of these materials to:

• Obtain a U of M Biosafety Permit before beginning work.
• Ensure lab personnel are trained in the procedures and training is documented. A training matrix is included. See Appendix A.
• Ensure your Biosafety Permit inventory lists the plasmids in use and also lists the assembled viral vector ("viruses") as a separate item if it is used or stored as such.
• Ensure a biological agent specific and/or at a minimum the Campus-specific U of M Post-Exposure Protocol (PEP), is posted and all personnel are aware of and can initiate the requirements including providing sufficient information to a health care provider in case of exposure.
• Develop any additional site-specific protocol as per your local risk assessment (LRA) on the specific procedures and construct in use. Where the work will be done in a multi-PI lab the risk assessment should involve all the PIs or the local lab safety/management committee.
• Lentiviral systems that are different than the one described at the bottom of page three (3) may have a different LRA. This may necessitate potentially different/additional safe work practices and a Biosafety Project Approval by the Biological Safety Advisory Committee (BSAC).

D. BACKGROUND
Self-inactivating, replication-incompetent VSG-G pseudotyped lentiviral vectors have become very widely popular in the manipulation and functional analysis of a protein of interest. This has resulted in their wide availability commercially and through custom services such as the Faculty of Medicine Lentiviral Vector Viral Particles Production Core Platform. These features have also resulted in their use by researchers without extensive microbiological training. This SOP is intended to provide a standardized guidance for all researchers working with these agents at the U of M. This risk assessment starts by looking at the wild type virus. The Public Health Agency of Canada (PHAC) HIV Directive (January 2014)
January 2014 the Public Health Agency of Canada (PHAC) released a directive on the containment level requirements for work with wild type virus HIV. (http://www.phac-aspc.gc.ca/lab-bio/res/bio-dir-htlv1-eng.php#a1_0) While this HIV Directive continues to support the classification of (wild type HIV-1) as a Risk Group 3 (RG3) agent due to its ability to produce serious disease, the outcome of a revised risk assessment indicates HIV (and HTLV-1) can be safely handled at Containment Level 2 (CL2) with specific additional operational requirements and biosafety considerations. The directive indicates that the derogation of the containment level requirements is supported by assessments that report:

• transmission is largely dependent on parenteral inoculation and contact with mucous membranes;
• survival of the virus outside the host is limited and occurs only under ideal conditions;
• airborne transmission is not possible; and the risk for laboratory users as well as the environment is limited;
• culturing (propagation) or in vivo work involving either pathogen does not increase the risk for the laboratory user and that proper handling and operational practices can mitigate the risk.

Section 4 and Section 5 of the HIV directive details the additional requirements and biosafety considerations that require careful consideration in the development of the local risk assessment (LRA) and Standard Operational Practices (SOPs) in order to work at CL2/CL2-Ag. These items have been taken into
careful consideration in the development of the operational standards and safe work practices found in Section 3 of this document.

**Additional Resources**
The References Section at the end of this document (Appendix D) provides a selection of excellent references on the safety of lentiviral vectors. Everyone working with these agents should familiarize themselves with a broader discussion of the hazards than that found exclusively in this SOP.

In reviewing those references, one clarification that may be required here is that many of these documents refer to Containment Level 2 (CL2) and CL 2+ practices (CL2 facility design with CL3 operational practices) which is language perhaps more in the context/reflective of the old PHAC Laboratory Biosafety Guidelines 3rd edition. Many of the requirements in the CBSG 1st Edition (2013)/CBS 2nd Edition (2015) are **risk and performance-based** and as such, are **dependent on the local risk assessment (LRA) performed**. In other words, there isn’t one set of prescriptive ‘standard’ CL 2 practices and another set of standard CL2+ practices.

As stated above, all of the CBSG CL2 operational standards and HIV Directive additional requirements and biosafety considerations have been taken into account in the development of this SOP and can be found in Section 3 of this document. Where warranted, items that would require an additional local risk assessment are highlighted or a discussion of the recommendation is provided.

**Section 2. PATHOGEN RISK ASSESSMENT - Related to lentiviral vectors**

**General Overview**
Lentiviral vectors are commonly used in the gene transfer applications because of their ability to infect both dividing and non-dividing cells, and also hard-to-transfect primary cells. They are designed based on the Human Immunodeficiency Virus (HIV)-1. Refer to the [HIV Pathogen Safety Data Sheet](#) for an overview of the risks associated with the wild-type virus. Later generations have been modified to remove all the replication and accessory genes in order to provide a safer version of the replication-competent virus particles to be used in transductions. Substitution of the native envelope with the G envelope glycoprotein of vesicular stomatitis virus (VSV-G) provides virions with a larger host range, greater infectivity and higher stability.

The NIH Recombinant DNA Advisory Committee (RAC) Guidance Document “**Biosafety Considerations for Research with Lentiviral Vectors**” indicates the major risks of research with HIV-1 based lentivirus vectors are the potential for generation of replication-competent lentivirus (RCL) and potential for oncogenesis. The document further indicates that these risks can be reduced by the nature of the vector system (and its safety features) or increased by the nature of the transgene insert encoded by the vector. These would provide the potential for increased pathogenicity or the potential for sero-conversion even with non-replicating viruses. The diagram at the bottom of page 4, taken from the NIH RAC Guidance Document, provides a good overview of the relative (higher or lower) risk with different vector systems.

**Specific Pathogen Description and Risk Assessment**
This procedure is specific to 3rd (or later) generation lentivirus vector systems. Changes from the parent HIV-1 would include:
- A critical number of genes have been removed (e.g. they do not encode Tat, which is essential for replication of wild-type HIV-1)
- Separation of the vector and packaging functions onto three or more plasmids
- The native HIV-1 envelope protein has been replaced, by a heterologous coat protein such as VSV-G.

While all of these engineered safety features are likely to provide for a greater margin of personal and public safety than earlier vectors, the virus is still capable of completing one cycle of integration into the host genome. Additionally, while the ability of the packaging cell line to provide the rescue mechanisms for
creating a replication-competent lentivirus (RCL) is considered to be very low, this cannot be totally discounted.

In summary, the primary hazards can be considered to be:

- Potential for generation of replication-competent lentivirus (RCL).
- Potential for insertional mutagenesis and deletions/insertions which may require additional precautions. (e.g. an insert that codes for a toxin, activates oncogene or inactivates tumor suppressor genes)
- Vector titer and volume (as these increase, so does the risk of exposure).
- Ability of the vector to transduce a wide range of cells.
- Potential for sero-conversion, even with a non-replicating virus, leading to a false positive HIV test result.
- Failing to understand that safety-engineered and commercially available lentivirus vectors are still Risk Group 2 agents and still require compliance with CL2 facility design or operational practices outlined in this guidance document.

* This can occur if a personal exposure occurs during the step where the virus is reassembled in the packaging cell line.
**This can occur in the event of a personal exposure at any time after the virus has left the cells and is in the supernatant.

Primary exposure/transmission routes in the research lab include:

- Puncture injury from contaminated needles or other sharp objects
- Spills and/or splashing of samples resulting in direct or indirect exposure of
  - non-intact skin such as scratches, cuts, abrasions, dermatitis or lesions.
  - mucous membrane including the eyes, nose and mouth.

These factors would indicate that the Risk Group of the described lentivirus vector constructs should still be considered Risk Group 2 and require strict adherence to the Containment Level 2 facility design and safe work practices listed.

### Section 3. CONTAINMENT LEVEL 2 (CL2) REQUIREMENTS
The Public Health Agency of Canada’s (PHAC) Canadian Biosafety Standards (CBS) Second Edition’ CL2 requirements include both Facility Design and Operational Standards. The safe work practices described here in Part B, are adapted from the CBS and HIV directive.

A. FACILITY-DESIGN STANDARDS

Refer to all of the CL2 ‘Facility Design’ requirements in Chapter 3 of the CBS 2nd edition. Note the following:

- Definitions for Containment Zone and the requirement for a closed (lockable) door and signage at the entrance to the Containment Zone (CBS 3.3.1-3.3.2).
- Space at the entrance to the Containment Zone for storage of personal protective equipment (CBS 3.3.9).
- Vacuum systems to be equipped with a mechanism that prevents internal contamination (3.7.17).
- Two-way communication system(s) inside the containment barrier that allows communication between inside the containment barrier to outside the containment zone, in accordance with function (3.7.18).

B. OPERATIONAL STANDARDS

Refer to all of the CL2 General Work Practices listed in Chapter 4 of the CBS 2nd edition. The items that follow are highlighted specific to this guidance document.

The items have been designated M=Mandatory or R=Recommended. The ‘Mandatory’ designation is either because:

- this is a required element under the CBS that we would like to highlight. OR
- the item has been designated as mandatory for this U of M Biosafety Program risk assessment.

NOTE: Until all the lentivirus vector or particle containing material has been inactivated appropriately, all of the following safe work practices apply to the handling of these materials. Inactivation may include disinfection or decontamination steps (e.g. autoclaving or inactivation using heat or another chemical that is part of the experimental procedure.

1. Biosafety Program Management (CBS 4.1)

M  Local Risk Assessment:

CBS 4.1.8 A local risk assessment (LRA) to be conducted to examine each task involving infectious material or toxins so that the risks are identified and safe work practices developed and documented.

This document provides the basis for a local risk assessment (LRA) for the construct specified in Section 2, on page 3. Each Containment Zone/Unit where this work is done should review the items and develop and append their own set of additional site-specific practices/SOP as required.

For example, depending on the number of rooms and people working in a containment zone you may, as an example, need to additionally define locations for the work, protocol for use of shared equipment and additional awareness training for personnel having access to the space but not working with these items.

Look for ‘LRA’ to identify additional items and discussion topics under the remaining topics as well.
2. Medical Surveillance Program (CBS 4.2)

Before starting work with lentiviral vectors you need to know:
If you are immune-compromised or have a pre-existing infection with HIV you are potentially at greater risk of more serious consequences after an exposure. If you meet these criteria or have concerns, you should discuss this SOP and your concerns with your personal health care provider.

M Post-Exposure Medical Assistance and Reporting

CBS 4.2.2: Containment zone personnel to immediately inform appropriate internal personnel or authority of any: incident that may have resulted in an exposure of an individual to a human pathogen or toxin in a facility; or disease that may have been caused by an exposure to a human pathogen or toxin in a facility.

Notification of any major spills or actual or potential exposures must be reported to your supervisor/permit holder as soon as possible. This should not delay initiating spill clean-up or obtaining medical assistance first, if required, as this would be the priority. For detailed information on response and reporting requirements, refer to the Biological Agent Incident Response & Reporting Guidelines. The basic steps follow:

1. Follow the first aid procedure on the U of M Post-Exposure Protocol (PEP). Seek medical advice within 2 hours and ensure you take along the PEP and Appendix D Emergency Medical Card.

2. Ensure that you file a 'Notice of Injury' green card with your supervisor and a copy to EHSO, both within 24 hours.

3. Employees must additionally complete a Worker’s Compensation Claim by calling (204)-954-4100 if the incident includes a lost-time injury or requires medical assistance.

4. For lost-time injuries or those requiring medical assistance supervisors are required to complete and submit the Incident Investigation Form to Judy.Shields@umanitoba.ca within 24 hours. Assistance with this can be requested.

3. Training Program (CBS 4.3)

M The PI/permit holder is to provide training on this SOP, first aid and Post Exposure Protocol and any pertinent site-specific conditions. A sample training matrix is found in Appendix A. In addition, in shared/open area lab spaces, all personnel who have access to the containment zone, must have a working knowledge of the location and hazards of ALL of the work being done in the space. This training can be developed in conjunction with the local safety/lab management committee.

CBS 4.3.8 Trainees to be supervised by authorized personnel when engaging in activities with infectious material and toxins until they have fulfilled the training requirements.

CBS 4.3.6 Visitors, maintenance and janitorial staff, contractors, and others who require temporary access to the containment zone to be trained and/or accompanied in accordance with their anticipated activities in the containment zone.

4. Personal Protective Equipment (CBS 4.4)

M Laboratory dress code: covered legs, arms, feet.
PPE are to be available at the entrance to the lab and removed before exiting. For work with lentiviral vectors, individual containment zones may need to develop specific donning and doffing procedures.
Gloves
- **Double gloves with the outer layer covering the sleeves of the lab coat are mandatory for all work with lentivirus vectors inside the BSC.** Remove the outer layer before leaving the BSC. Inspect gloves for tears and punctures before and after putting them on.
- Do not touch contaminated surfaces with bare hands when removing your gloves.
- Wash your hands immediately after removing gloves.
- Always remove gloves before touching clean surfaces in the lab such as a phone, computer, light switch, door handle or book.
- Gloves that have been in contact with biohazardous material should be considered contaminated and should be decontaminated before disposal. Refer to the [U of M Biohazardous Waste Chart](#).

Lab Coats
Lab coats dedicated to the containment zone and worn fully buttoned, are mandatory. If any clothing is exposed or suspected of exposure, decontaminate the clothing before laundering. For example, autoclave the lab coat or treat the contaminated area with bleach.

Lab coats with cuffs are recommended.

**LRA** - Consider a back-closing gown worn over the regular lab coat dedicated to the work, or an additional different lab-coat, dedicated to and stored at the BSC location. Options are back-closing gowns or removable sleeves over lab coat. *(Note: the HIV directive requires an additional layer of protective clothing to be donned prior to work with infectious material in accordance with entry procedures.)*

Face Protection
The primary route of exposure is through broken skin or mucous membranes. While working in the BSC, the sash provides a barrier between the worker and agent. Personnel may additionally choose to wear safety glasses, face shields or surgical masks during all procedures with lentiviral vectors including work in the BSC. This would only prevent accidentally touching these areas while working. Surgical masks DO NOT provide respiratory protection.

In addition to all other PPE, full face protection (full face shield; or safety glasses and mask) is mandatory for any work with lentivirus in open containers outside of the BSC. For example during a spill clean-up.

5. **Entry and Exit of Personnel, Animals, and Material (CBS 4.5)**

Access, Authorization, Entry and Exit to Containment Zone

*CBS 4.5.1* - Containment Zone doors to be kept closed.
*CBS 4.5.2* - Access to the containment zone is limited to authorized personnel and authorized visitors. **CBS definition of ‘Authorized’: An individual who has been granted access to the containment zone by the Containment Zone director, and/or another individual to whom this responsibility has been assigned. This is dependent on completing training requirements and demonstrating proficiency in the SOPs, as determined to be necessary by the facility.**

*CBS 4.5.10* - Personal clothing to be stored separately from dedicated PPE.
*CBS 4.5.11* - Personal belongings to be kept separate from areas where infectious material or toxins are handled or stored.
*CBS 4.5.14* - Personnel to doff dedicated PPE in a manner that minimizes contamination of the skin and hair when exiting the containment zone.
*CBS 4.5.15* - Personnel to remove gloves and wash hands when exiting the containment zone.
*CBS 4.5.8* - Current entry requirements to be posted at point(s) of entry to the containment zone.
A **U of M Workplace Hazard Information Placard (WHIP)** with current emergency contact info, CL2 and biohazard logo is required.

### 6. Work Practices (CBS 4.6)

**Reminder:** All of the Containment Level 2 General Work Practices listed in Section 4.6 of the [CBS 2nd Edition](#) are to be followed. The items that follow highlight specific practices of note.

| M | Good microbiological practices to be employed (CBS 4.6.18). Refer also to the [PHAC poster](#). Appendix B of this document has a further list of exposure control practices based on CBS 4.6. |
| M | Open wounds, cuts, scratches, and grazes to be covered with waterproof dressings. (CBS 4.6.6) |
| M | **All manipulations of the virus must be done within a certified Biological Safety Cabinet (BSC)** according to the ‘Proper Use’ instructions found in the [CBSG 1st Edition](#), Part II, Chapter 11.4 or in the [PHAC e-learning modules](#). (HIV Directive) |
  
  Critical item: Adjust the stool height so that your underarms are level with the bottom of the sash. Do not continue if your face is not protected by the sash of the BSC. |
| M | All waste must be collected in the BSC. Before removal from the BSC, the contaminated material must either be fully decontaminated, or, packaged and the package surface disinfected in the BSC before removal for autoclaving. Refer to Section 3 B.7 for disinfection information. |
| R | For shared equipment areas: In conjunction with your local lab/safety committee, consider labelling all fridges, freezers, incubators, centrifuges used with these biological agents as biohazardous. For example: When spinning down cells that have been in contact with virus, or washing lentiviral preparations, take the following precautions: |
  | o Put a sign on the centrifuge noting the work in progress. |
  | o Remember to remove the sign once you have disinfected the centrifuge. |

### 6.1 Procedures Where Sharps Are Prohibited (based on this LRA)

| M | Plastic-ware should be used instead of glassware. |
| M | **Needles/Syringes,** glass pasteur pipettes, **blades** or any tools that may reasonably expected to puncture the skin, are NOT permitted in the BSC when lentivirus work is being performed; especially when lentivirus is being produced by packaging cells or during any activity when lentivirus particles or virions are present. Use of these sharps or any potential sharps after lentivirus work in the BSC may only take place when the BSC has been thoroughly decontaminated. Refer to Section 3 B.7 for guidance on disinfection and decontamination. |

### 6.2 Use of Aspirators

| M | **DO NOT use an in-house vacuum system or a local vacuum pump as part of an aspiration system while working with the live lentivirus.** For example, the removal of culture media or vessel supernatant containing or potentially containing this material can be performed by drawing up the solution using a pipette-man or automatic pipettor tip or pipette (with filter/plugs) and carefully disposing the solution/suspension in a waste beaker with an appropriate final concentration of disinfectant. Glass Pasteur pipettes are NOT permitted at any time. Plastic pipettes specifically for this purpose are available and required. |

| M | If you choose to use an in-house or local pump vacuum system for aspiration of non-infected cell culture supernatants or clean media, the following are still required: |
At a minimum, a double flask set-up, positioned in secondary containment is required.

- A hydrophobic & aerosol (HEPA filter) vacuum protection device must be installed in the vacuum line shortly after the second collection flask. Filters must be autoclaved before disposal.

### 6.3 Use of the Centrifuge

**M** All centrifugation must be done in centrifuges equipped with sealed centrifuge rotor cups or safety buckets that are loaded, closed and opened in the BSC. (HIV directive) Disinfect the surface of the rotor before removing it from the BSC. *(For example: Prepare your cells and add to screw top centrifuge tubes. Place tubes in the buckets and put the cover on the bucket ensuring that it is well seated and secure. Make sure the rubber seal on the lid is intact and in place. Do not spin down cells if the lid assembly is compromised). At the end of the spin carefully open the lid (of the centrifuge or rotor) and look for potential leaks. If everything looks good take the assembly to the hood and carefully remove contents ensuring that there are no leaks. If you see a leak, close the lid and wait 30 minutes and then proceed as per your local spill protocol.)*

**M** Once your work is complete, wipe down the bucket assembly with an approved disinfectant both inside and outside and let dry.

### 6.4 Transport of lentivirus-infected cells and lentiviral particles within or between containment zones

**CBS 4.6.31-** Procedures as determined by a LRA to be in place to prevent a leak, drop, spill, or similar event during the movement of infectious material or toxins within the containment zone or between containment zones within a building.

**M** At a minimum, lentivirus material should always be contained in break-resistant (plastic), securely sealed, screw-top primary containers (e.g. test tube, tissue culture flask) and transported within Containment Zones in such a manner that the viruses are contained in the event they are dropped or turned over. Containers should have been surface disinfected before removing from the BSC.

**M** Lentivirus material and any contaminated waste material transported between containment zones must be contained in securely sealed primary containers within secondary containers with a secure lid. At a minimum, secondary containers should be surface disinfected before leaving the containment zone and again after removal of the item at the second location. Transport between containment zones on different floors should be done using a transport cart and service elevators versus public elevators (where available).

**R** A small spill kit can be prepared and kept on the transport cart, making it available at the source, during transport, and at the destination.

### 6.5 Storage of lentivirus-infected cells and lentiviral particles inside or outside the containment zone

**M** Typically for short-term storage inside the containment zone, virus can be stored at 4°C for 24-48 hours in a screw cap container and the container sealed with Parafilm. For long term storage, lentivirus preparations should be aliquoted in cryovials and kept at -80°C and inside a secondary container. In both situations, make sure the specimens/containers are well labeled with direct user’s name and the Permit holder holder’s name, and contents are clearly identified. The preparations should be stored in a secure containment zone/equipment.

**M** At the U of M, when storage is in freezers located outside of the containment zone, or in public access hallways the equipment must be locked and be labelled with signage that contains contact information similar to that on a WHIP sign. Contents must be monitored regularly and stored
items must be included in the permit holder’s EHSA database biological agent inventory as part of their Biosafety Permit information. **Note:** this is included to mean that all lentivirus material including waste, must be left secured at all times. e.g. No storage of biohazardous waste in hallways outside of a containment zone.

**CBS 4.6.20** - Containers of infectious material or toxins stored **outside the containment zone** to be labelled, leakproof, impact resistant, and kept either in locked storage equipment or within an area with **limited access**.

**Limited access (definition from CBS):** Access that is only permitted to authorized personnel and other authorized visitors through either operational means (e.g., having authorized personnel actively monitor and check all individuals entering a designated area) or through the use of a physical barrier (e.g., a controlled access system, such as, key-locks, or electronic access card).

### 6.6 Use of Incubators

**R** Ideally, all cultures should be grown in vent top flasks.

**M** In cases where standard tissue culture plates are used these must be kept in sealable plastic containers (or any similar tray container) to contain any potential spill of the tissue culture medium. Once inside the incubator, container lids can be opened to allow for gas exchange. It is extremely important to ensure that your dishes and/or plates clearly indicate the presence of virus.

**R** A label on the incubator when this work is in progress may be indicated as well. Some areas may also have the ability to have an incubator dedicated to lentiviral work.

### 7. Decontamination and Waste Management (CBSG 4.8)

**M** Biohazardous Waste Disposal: General

- Post and follow the U of M **Biohazardous Waste Disposal Chart**. For a more complete discussion of the requirements refer to the section on the disposal of Biohazardous Waste in the U of M Biosafety Guide.

**Decontamination and Disinfectants:**

**M** All infectious material (including cell-associated or cell-free virus suspensions and supernatants) and other contaminated waste used or generated inside the BSC **must be decontaminated inside the BSC** or the **items can be packaged and the packaging can be surface disinfected before removal** and final decontamination by autoclaving. A selection of concentrations and methods for chemical disinfection and decontamination are discussed in the table below.

**M** When you are finished working in the BSC and all equipment and packaged items have been surface disinfected and removed, disinfect all interior horizontal and vertical interior surfaces of the BSC selecting from the concentrations and methods discussed below.

<table>
<thead>
<tr>
<th>Purpose</th>
<th>Product Description</th>
<th>Preparation</th>
<th>Contact Time</th>
<th>Additional information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decontamination of contaminated liquid (e.g. cell associated or cell-free virus suspensions or supernatants, etc.)</td>
<td>final concentration 5000 ppm sodium hypochlorite (e.g. Clorox™), bleach has a limited shelf life</td>
<td>e.g. To a 500 ml volume container add 50 ml commercial bleach (5.25-6% sodium hypochlorite) then add liquid waste to the container.</td>
<td>30 minutes</td>
<td>Tightly close and surface disinfect the waste bottle/container before removing it from the BSC. After the appropriate incubation time the incubated liquid can be discarded down the sink with large quantities of water. <strong>Caution:</strong> Do not autoclave! Do not top up an old solution with fresh product; prepare a fresh waste bottle daily/prior to use.</td>
</tr>
<tr>
<td>High titer spills</td>
<td>10000 ppm sodium</td>
<td>1:5 dilution of commercial bleach</td>
<td>30 minutes</td>
<td>Follow spill guide guidelines in Section 3. B.9 of this document and at the following links:</td>
</tr>
<tr>
<td>Surface Disinfection</td>
<td>5000 ppm sodium hypochlorite (Bleach- e.g. Clorox™)</td>
<td>1:10 dilution of commercial bleach (5.25-6%) made fresh weekly; preferably daily.</td>
<td>10 minutes</td>
<td>Caution: May corrode metals. If using on stainless steel surfaces, after the appropriate contact time, follow with a water and 70% ethanol rinse of all bleached areas.</td>
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</tr>
<tr>
<td>Surface Disinfection</td>
<td>70% Ethanol</td>
<td>Not Recommended</td>
<td>See comments</td>
<td>While enveloped viruses such as HIV are typically regarded as effectively inactivated by many chemical means, the literature reports vary regarding the efficacy of 70% ethanol for the disinfection of HIV virus. Efficacy appears to be related to whether the virus is cell-associated, in the presence of high organic loads, titer, in suspension and/or dried to a surface. Therefore 70% ethanol is not recommended as a standard method of surface disinfection for HIV unless the surface has been effectively pre-cleaned and a 10 minute contact time can be maintained. Since ethanol readily evaporates and is flammable this is usually difficult to achieve.</td>
</tr>
<tr>
<td>Surface Disinfection</td>
<td>0.5 % accelerated hydrogen peroxide e.g. Accel TB™, Oxivir TB™</td>
<td>Follow manufacturer’s instructions.</td>
<td>Follow manufacturer’s instructions.</td>
<td>Note: Be sure to read and follow all manufacturer’s instructions. 1- Some manufacturers have similar products which work with different contact times. 2- Some products are only effective if surfaces are pre-cleaned. E.g. surfaces must be wiped twice, ensuring the second application sufficiently wets the surface for the indicated contact time.</td>
</tr>
<tr>
<td>Surface Disinfection</td>
<td>Quaternary Ammonia Compound (QAC)( e.g. Cavicide™)</td>
<td>Follow manufacturer’s instructions.</td>
<td>Follow manufacturer’s instructions.</td>
<td>If product label indicates these are EPA approved for HIV or in Canada labelled as virucidal for HIV, these may be an appropriate disinfectant for some uses. Similar to the hydrogen peroxide based products, these usually require a pre-cleaned surface/ wiped twice. QACs have detergent features therefore contributing to surface cleaning. Many are formulated with two different QACs or one QAC and an additional percentage of ethanol.</td>
</tr>
</tbody>
</table>

**Disposal of solid waste inside the BSC:**
- All solid waste used inside the BSC must be collected/contained inside the BSC or decontaminated before removal.
- Smaller autoclave bags can be used for gloves, paper towels etc. Where limited space restricts the amount of waste that can be contained, contaminated items that are free of liquid and closed securely (e.g. tissue culture flasks), can be surface disinfected and then removed to an autoclave bag immediately outside of the BSC. Preferably this should be done at the end of the work but if this is not possible, attention should be given to strictly limiting the number of arm movements in and out of the BSC.
- All disposable pipette tips, serological pipettes etc. should be collected in an autoclavable, rigid, puncture resistant, container with a lid. DO NOT FILL THE CONTAINER MORE THAN ¾ FULL. Disinfect the surface of the container with the appropriate concentrations of ethanol or bleach prior to removal from the BSC.
- Solid wastes (tissue culture flasks) previously disinfected inside the hood with bleach should not be autoclaved. They can then be disposed inside a clear plastic autoclave bag outside the hood.
**Autoclaving waste:**
- Add autoclave tape to the container/bag to indicate the processing status. Autoclave for a minimum of 1 hour at 121°C. Refer to the Biohazardous Waste Disposal Chart for more details.
- All solid, contaminated waste should be autoclaved at the end of the procedure or by the end of the work day. If overnight storage of contaminated waste is unavoidable, the contaminated waste (autoclave bags, sharps containers) must be placed in well labelled, disinfectable containers with a securely closed lid. Autoclaving should then be done as soon as possible the next day.

### 8. Documentation and Records

**Inventory Recording and Inventory monitoring**
- CBS 4.10.2 Inventory of infectious material and toxins handled in the containment zone or stored in/ outside the containment zone to be maintained, and kept up to date.
- HPTA Section 14 Personnel are required to notify their PI or the lab supervisor if they are aware of a known loss of material or feel that security surrounding this work is threatened. The PI must notify the BSO of any known or suspect loss of inventory. Refer to Biological Agent Incident Response & Reporting for additional detailed U of M procedures.

### 9. Emergency Response Planning (CBS 4.9)

Refer also to the U of M Emergency Quick Reference Guide.

#### 9.1 Medical Emergencies

For on-campus accidents that are life threatening, contact emergency personnel immediately:
- University phones: 911 or 4-911 or 555 or
- MTS/Rogers cell phones #555 or 911
- Other available phones located throughout campus include Red Emergency Phones; Classroom Emergency Phones; Elevator Emergency Buttons & Code Blue Stations

#### 9.2 Personal Exposures

Exposure Protocol includes not only a puncture wound due to a contaminated sharp instrument, but also exposure by way of a splash of hazardous chemical or biological agents into the eyes, mouth or non-intact skin including bites and/or scratches.

#### 9.3 First Aid

If a known or suspected exposure occurs to:
- **Eyes and other mucous membranes or non-intact skin** - flush with water for 15 minutes. Use the safety shower for a splash to a large area of exposed skin.
- **Puncture Injury** - encourage bleeding of injury site - wash injury site thoroughly with soap and water - cover area with sterile dressing if necessary
- **Any small intact areas of skin**: initiate first aid by washing skin surfaces thoroughly and immediately with soap and water.
- **Large skin areas**: activate the emergency shower.
- **Eyes**: use the eye wash according to directions to thoroughly flush the area.

#### 9.4 Post Exposure Protocol and Reporting

1. Report incident to supervisor or department as available.
2. **Seek medical assistance within two hours**. Take along the U of M Post Exposure Protocol, and Appendix 2 and any other available MSDS or lab-specific information if available.
3. Ensure that you file a ‘Notice of Injury’ green card with your supervisor and a copy to the Occupational Health Coordinator at EHSO within 24 hours.
4. Employees must additionally complete a **Worker’s Compensation Claim** by calling (204)-954-4100 if the incident includes a lost-time injury or requires medical assistance. In this case, the supervisors are required to complete and submit the **Incident Investigation Form** to Judy.Shields@umanitoba.ca within one week of the incident. The Biosafety Program will assist with all incident investigations involving biological agents.

5. For detailed information on response and reporting requirements, refer to the **Biological Agent Incident Response & Reporting** SOP.

9.5 Spill Clean-up
Refer to **PHAC Spill Clean-up Job Aid**. Use the following definitions for minor and major spills.

**Minor Spills:**
Small spill inside the BSC:
- < 20 ml of unconcentrated virus (1 x 10^6/ml) or
- < 0.5 ml of concentrated virus (1 x 10^8).

**Major Spills:**
- > 20 ml of unconcentrated virus or > 0.5 ml of concentrated virus in the BSC or ANY spill outside of the BSC.

**General Spill response (PHAC Spill Clean-up Job Aid)**
1. Have suitable disinfectants available in the lab at all times. Refer to the previous section on disinfectants.
2. Notify people in the vicinity and ensure everyone’s personal safety.
3. Wear all the PPE indicated in this SOP including double gloves and full shoes and cover your legs. In addition, wear a back-closing gown and full-face protection when cleaning up any spills outside of the BSC.
4. Basic steps are:
   a. Allow aerosols to settle
   b. While wearing protective clothing, gently cover the spill with paper towels.
   c. Apply an appropriate disinfectant, starting at the perimeter, working inwards towards the centre.
   d. Use a gentle flooding action to reduce the creation of aerosols.
   e. Allow sufficient contact time before clean-up.
   f. Use forceps to pick up any broken glass or sharps and place them in a leak-proof puncture resistant container.
5. Move carefully during spill clean-up to avoid splashes and/or self-contamination.
6. If a known or suspected exposure occurs to any clothing, treat that item as contaminated. It must be decontaminated by soaking in bleach or autoclaving before laundering.
7. Report spills, accidents or exposures as soon as possible to the laboratory supervisor/PI. A written record of such incidents must be maintained, and the results of incident investigations should be used for continuing education. Refer to the **Biological Agent Incident Response & Reporting** SOP.

9.6 Loss of containment (e.g. electrical power failure)
1. In the event of a loss of containment such as a power outage while lentiviral work is in progress, all work shall be contained and stopped as soon as possible. Close all containers securely or
decontaminate and disinfect material that cannot be contained. Leave your gloves in the BSC. If the BSC has a closable sash, close until containment/power has been restored.

2. Post a sign on the BSC indicating the work in progress and to keep the sash closed or stay out until power is restored.

3. If there is a power failure during a centrifuge run, if possible, wait until the centrifuge stops and remove samples/rotor to the BSC and then remove your gloves and close the sash.

4. If you need to leave the CL2 zone, use proper exiting procedures.

5. If you choose to, or need to relocate your experimental materials to another location or CL2, all transport must be as described previously. In Brief: transport must be in secondary puncture/leak resistant containers with a secure lid. At a minimum, containers should be surface disinfected before leaving the lab and again when emptied. Transport between floors should be done using service elevators (where available) and carts for larger loads.

9.7 Fire Response

1. In the event of a fire alarm you must leave the building (U of M Policy). Before any work begins, supervisors should determine the most appropriate steps to ensure the integrity of their experiments and discuss specific details/scenarios with their lab personnel. Then in the case of a fire alarm no one will panic and everyone will confidently take the appropriate steps to protect themselves and their research.

2. Basic Principle: If a fire alarm sounds while lentiviral work is in progress, all work shall be stopped and contained as soon as possible as time and personal safety permit. Close all containers securely. Leave your outer gloves in the BSC. Lower the sash if your BSC has that option. Do not wait for the centrifuge to stop. Remove the rest of your PPE and use a hand sanitizer if possible before leaving the Containment Zone or exit the lab according to your local fire response exit plan and proceed to your designated meeting area outside of the building.

3. Fire safety response plan as per the info at the U of M Emergency Quick Reference Guide and in brief below.

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**FIRE SAFETY**

**IF YOU DISCOVER A FIRE OR HEAR THE FIRE ALARM**

1. **IMMEDIATELY** activate the nearest fire alarm pull station.

2. At your discretion, attempt to control the fire with available fire equipment.

3. Remain calm and leave the fire area via the nearest safe exit.

4. Close doors behind you when exiting.

5. **DO NOT USE THE ELEVATOR**

6. Dial 555 from a university phone, #555 on any Rogers or MTS cellular, or dial 4-911 from a university phone or 911 from any other phone. Tell them you are reporting a fire at: _______ state your location _______

7. When you have reached the outside, move away from the building.

8. Go to the designated assembly area.

9. **DO NOT GO BACK INTO THE BUILDING FOR ANY REASON**. The Fire Department will advise when it is safe to do so.

All staff, students and visitors should be familiar with the Fire Safety Procedures and related Appendices found on the University of Manitoba’s Governing Documents page at: [http://umanitoba.ca/admin/governance/571.html](http://umanitoba.ca/admin/governance/571.html)
Appendix A: Sample Training Matrix for Work with Lentiviral Vectors

To be used in conjunction with the Laboratory Safety Checklist for New Lab Personnel. This checklist can augment Question 2b: The Supervisor/PI has discussed hazardous components of the research with biological hazards. For this SOP on lentiviral vectors, lab personnel must demonstrate proficiency in the practices and operations of the lab facility for the following.

Date | Site and Procedure Specific Training
--- | ---

**General Safety**
- □ Departmental WHMIS training
- □ EHSO Generic Biosafety Training
- □ Biosecurity, access and inventory control

**Lentiviral Knowledge**
- □ Pathogen hazards and signs and symptoms of infection
- □ Modes of transmission
- □ Hazardous procedures
- □ Biosecurity, access and inventory control

**Personal Protective Equipment (PPE)**
- □ Lab dress code
- □ Availability, location and proper donning and removal of lab coats/gowns, gloves, eye/face protection.
- □ Disinfection of PPE in case of spill or other exposure

**Exposure Control Work Practices**
- □ BSC Use
- □ Handling and disposal of sharps
- □ Safe use of centrifuges and autoclave (as applicable)
- □ Pre and post-exposure clean-up procedures
- □ Hand-washing: where, when and how
- □ Storage and transport

**Decontamination/Disinfection & Waste handling**
- □ Appropriate disinfectants and their use
- □ Waste collection set-up and transport of biohazardous waste

**Emergency Response**
- □ Procedures for spills and leaks inside and outside of the BSC
- □ Post Exposure Protocol and incident reporting procedures
- □ Fire and Power failure procedures

The signatures below indicate that the above items have been discussed with the trainee and they have demonstrated the required proficiency to work independently with the lentivirus agent and procedures used in the lab.

____________________  ______________________
Trainee Signature           Supervisor/PI signature

Avoid exposure by Direct or Indirect Contact.

a. Working inside a BSC is mandatory.
   - Live viral vector preparations and transduced cells can only be handled inside a certified BSC.
   - Centrifuging can only be done using screw-topped tubes and/or sealed rotors or safety cups. Tubes/rotors can only be loaded, opened in the BSC.
   - All waste and equipment must be decontaminated inside the BSC or contained in an autoclave bag or tightly screw-capped container and surface disinfected before removal. Do not autoclave items containing bleach.

b. Wear double gloves (e.g. nitrile) and a lab coat at all times. Ensure all skin is covered; wear gloves over lab coat sleeves. Wear any other Personal Protective Equipment (PPE) prescribed in the document or by your PI.

c. Cover open wounds, cuts, scratches, and grazes with waterproof dressings and gloves. If you exhibit any open wounds (broken skin) in areas that cannot be covered by dressings or clothing, re-evaluate the work in process. Suggestions for mitigating the exposure in the case of broken skin that cannot be covered include, for example where the wound is on the face, consider doing the work in the BSC with an additional full face shield or safety glasses and surgical mask or have someone else do the work.

d. Keep hands away from the eyes, nose and mouth in order to avoid potential exposure of the mucous membranes.

e. Minimize splashes or aerosols with careful pipetting. Oral pipetting of any substance is prohibited in any laboratory.

f. In the event of a spill, contain and decontaminate the spill using appropriate techniques and materials.

g. Clean and decontaminate all work surfaces with a suitable disinfectant (1/10 dilution of household bleach) at the end of the procedure and after any spill of potentially biohazardous material.

h. Hand washing is a critical component of exposure control. Wash your hands after removing gloves and before leaving the laboratory and at ANY time after handling materials known or suspected to be contaminated.

i. Store all materials in screw-cap vessels, adequately labelled and in secondary leak-proof containers for short and long term storage.

j. Transport all materials containing lentivirus, including waste, in securely sealed, leak-proof containers within the containment zone and in additional sealed, puncture-proof secondary containers when outside of the containment zone.

k. Do not eat, drink or smoke or store food, personal belongings, or utensils in the lab AT ANY TIME.

l. Separate paperwork and report writing from work areas where biological agents are in use.

m. Do not wear protective laboratory clothing in non-laboratory areas.

n. Do not store laboratory clothing in contact with street clothing. Do not apply cosmetics, or insert or remove contact lenses. Wearing contact lenses is permitted only when other forms of corrective eyewear are not suitable.
o. Wearing jewellery is not recommended in the laboratory. For example, large rings can puncture gloves, and watches and necklaces can hang into and be contaminated by your cultures and thereby be a source of further environmental and personnel contamination.

p. Tie back or restrain long hair so that it cannot come into contact with hands, specimens, containers or equipment.

q. Decontaminate and label or tag-out contaminated materials and equipment leaving the laboratory for service or disposal. Follow the U of M Biohazardous Waste Disposal Chart, Decommissioning Procedure and the Standards of Care for Decommissioning Guidance Document.

**Avoid exposure by Accidental Puncture**

The use of sharps is prohibited for all work in the BSC while working with viral vector preparations and transduced cells and until the BSC is decontaminated after the work.

For all procedures replace glass with plastic where possible.
Appendix C: References

- Lentivirus Gene Engineering Protocols (Methods in Molecular Biology)” by Maurizio Federico, Publisher: Humana Press.
- University of Cincinatti, Viral Vector Web training modules: [http://researchcompliance.uc.edu/Biosafety/Training/ViralVectorWebtraining.aspx](http://researchcompliance.uc.edu/Biosafety/Training/ViralVectorWebtraining.aspx)
APPENDIX D: Emergency Medical Card

Each PI should develop an emergency response plan in case of exposure to lentiviral vectors. It is the responsibility of the PI to provide risk assessment information if emergency department providers have any questions regarding health hazards.

To the Health Care Professional:

I need to be evaluated within 48 hours. I have been working with a non-replicative viral agent based on the HIV-1 backbone. Changes from the parent HIV-1 would include:

- A critical number of genes have been removed to prevent replication in the body and the design of the virus greatly reduces the chance of generating a replication competent virus.
- The native HIV-1 envelope protein has been replaced by a heterologous coat protein (such as) VSV-G.

The primary hazards can be considered to be:

- Potential for generation of replication-competent lentivirus (RCL). (This is considered to be very low although samples have not been/are generally not tested for replication-competent virus.)
- The construct can infect the host cell once and deliver a genetic payload. There is a potential for insertional mutagenesis and deletions/insertions which may require additional precautions.
- As the vector titer and volume increase so does the risk of exposure.
- This viral construct can infect a wide range of cells.
- There is a potential for HIV positive sero-convension, even with a non-replicating virus.

The Insert in this vector is ______________________________.
The function of this Insert is expected to be ______________________________.

Workers shouldn’t be working with the constructs if they can’t answer this question. If the work in the lab includes work if a large variety of inserts, this information may be provided by the worker or supervisor at the time of the visit with the health care professional.

My supervisor/PI can be reached at: ____________________________.

A guesstimate of the exposure concentration is:

Unconcentrated virus usually ~1 x 10^6/ml) or
Concentrated virus can be ~ 1 x 10^8/ml

Reference- University of Cincinnati lentiviral vector training module:

They say that:

‘The current guidelines for managing occupational exposure to HIV-based viral vectors rely on the use of antivirals. In this type of exposure, antivirals should target the pre-integration steps of the viral cycle to prevent insertional risks. Ideally it is recommended that post-exposure prophylaxis (PEP) be administered immediately, but certainly no later than 72h following exposure.

Antivirals that inhibit the viral Reverse Transcriptase (RT) are use as PEP drugs. When RT is blocked, DNA copy cannot be formed from the viral RNA. Also inhibitors of the viral integrase may be added to the regimen.

PEP treatment needs to be prescribed by an occupational health physician who must determine whether treatment is necessary based on the biological hazards associated with the expressed transgene.’

Further they note however:

‘Each PI should develop an emergency response plan in case of exposure to lentiviral vectors. It is the responsibility of the PI to provide a risk assessment information if emergency department providers have any questions regarding health hazards.’