1. Introduction

Lentiviral vectors (**LVV**) are gene delivery systems derived from viruses that belong to the *Retroviridae* family. They are an effective system for the integration and sustained long term expression of transgenes of interest both *in vitro* and *in vivo*, and used by scientists to answer fundamental questions in biology and to create potential cures for genetic diseases (Schlimgen et.al, 2016).

Most LVV are derived from the human immunodeficiency virus type I (HIV-I) but can also be designed from other non-human lentiviruses like feline immune deficiency virus (FIV) and equine infectious anemia virus (EIAV) (Kost et al. 2006). They were created to overcome the limitations presented by murine leukemia virus (MLV) systems, being able to infect not only dividing but non-dividing cells. At the same time, they were engineered to be virus replication incompetent, and in some cases with self-inactivating properties.

The use of lentivirus presents some safety concerns. Although unlikely with most current LVV systems, there is some potential for reversion of the vector to a replicative state and the possibility of inadvertent generation of infective replication-competent retrovirus (RCR) as a result of multiple recombination events. LVVs also carry the inherent risks associated with the transgene, such as oncogenic and/or toxic effects. The presence of sequences with promoter activity raises the concern that insertional mutagenesis of the integrated provirus can result in the activation or inactivation of host genes with deleterious consequences to the host. There is clinical evidence that LVVs can cause clonal expansion and may cause oncogenesis through insertional mutagenesis when given in high dose to human subjects (Schlimgen et.al, 2016). In addition, some modifications, like pseudotyping, may increase cell tropism and the range of target cells that can be affected.

For these reasons, it is important to consider biosafety procedures and laboratory best practices to reduce the risk of exposure in laboratories handling and storing lentivirus.

2. Scope

This SOP is a guideline for completing a Pathogen Risk Assessment or Local Risk Assessment involving lentiviral vectors for in vitro and in vivo research

activities at Queen's University. Its content is in accordance with work practices recommendations described in the Canadian Biosafety Guideline – lentiviral vectors (PHAC, 2019) and the NIH Guideline – Biosafety Considerations for Research with Lentivirus (NIH, 2006). The procedure also describes physical and operational requirements for the safe handling of lentivirus vectors using a standard transient viral production method (Blesh, 2003) as well as required information to communicate to the Institutional Biohazard Committee (IBC) as part of the exposure response plan.

3. Applicable Legislation, Standards, Guidelines

Controlled activities with Lentivirus are done under the authority of the Public Health Agency of Canada's Human Pathogens and Toxins Licence issued to Queen's University – for Risk Group 2 human pathogens and toxins under section 18 of the Human Pathogens and Toxins Act (Queen's University Biosafety Manual 2021, pp24).
Canadian Biosafety Guideline – lentiviral vectors (PHAC, 2019).
Canadian Biosafety Standard 3rd Edition, 2019
NIH Guideline – Biosafety Considerations for Research with Lentivirus (NIH, 2006).

4. Responsibilities

4.1 Responsibilities of Department/Unit Heads

- Ensure that supervisors, employees, and students are notified about the provisions of this SOP, including their responsibilities for working with lentivirus vectors and their products.
- Support and assist, when necessary, Principal Investigators to ensure the components of this SOP and the applicable legislation are implemented in all facilities under the Head's authority.
- When necessary, assist the Department of Environmental Health and safety in addressing outstanding non-compliance issues that have been identified in a facility under the Head's authority.

4.2 Responsibilities of Supervisors

- PIs will be required to demonstrate in the Biohazard Permit Application the laboratory meets the physical and operational requirements to work with RG2/RG2+ lentiviral vectors.

- Develop laboratory specific procedures to work with lentivirus.
- Develop a laboratory-specific exposure response plan before an exposure incident.
- Ensure that all employees and students have received instruction in the proper procedure for handling lentivirus vectors.

4.3 Responsibilities of Users (Staff/Students)

- Be familiar with the content of this SOP.
- Complete the required safety training before any work with lentiviral vectors begins.
- Adhere to all safety precautions specified in this SOP.
- Report any issues, hazards, or concerns to a supervisor.

5. Definitions

Transfer/vector plasmid - contains the transgene flanked by Long Terminal Repeat sequences (LTR) and the virus packing signal (Ψ)

Helper (packaging) plasmid – contains the HIV structural (gag) and replication (pol) genes which code for the proteins required to produce the lentivirus particle and integrate the transgene. May contain regulatory/accessory genes, depending on generation. It is packing signal defective (-Ψ).

Helper (regulatory) plasmid – contains HIV regulatory and accessory genes (i.e. Rev), and is packing signal defective (-Ψ). Used in 3rd generation lentivirus systems.

Helper (envelope) plasmid – contains the HIV envelope gene that usually is replaced by another gene coding for a heterologous glycoprotein called pseudotyping.

First generation LV packaging system includes three plasmids derived from HIV-1 including standard retroviral genes and HIV specific genes. High titer stocks had a considerable risk of containing some replicative competent retrovirus (RCR) due to recombination events during virus production. (Fig1) Note: First generation systems are no longer permitted for use at Queen’s.

Second generation LV is a three-plasmid packaging system where HIV-specific accessory genes (except for Rev and Tat) have been removed from the Packaging plasmid, reducing the chance of RCR formation. (Fig 1)

Third generation LV is a four-plasmid system, where the packaging plasmid is split into two plasmids: One encoding structural (gag) and replication (pol) genes and another (helper plasmid) encoding the regulatory protein Rev. The requirement for Tat is eliminated through the addition of a chimeric 5'LTR fused to a heterologous promoter on the transfer plasmid, meaning that expression of the transgene is no longer dependent on Tat transactivation. With these modifications, there is a significantly reduced capacity of RCR formation. (Fig 1)

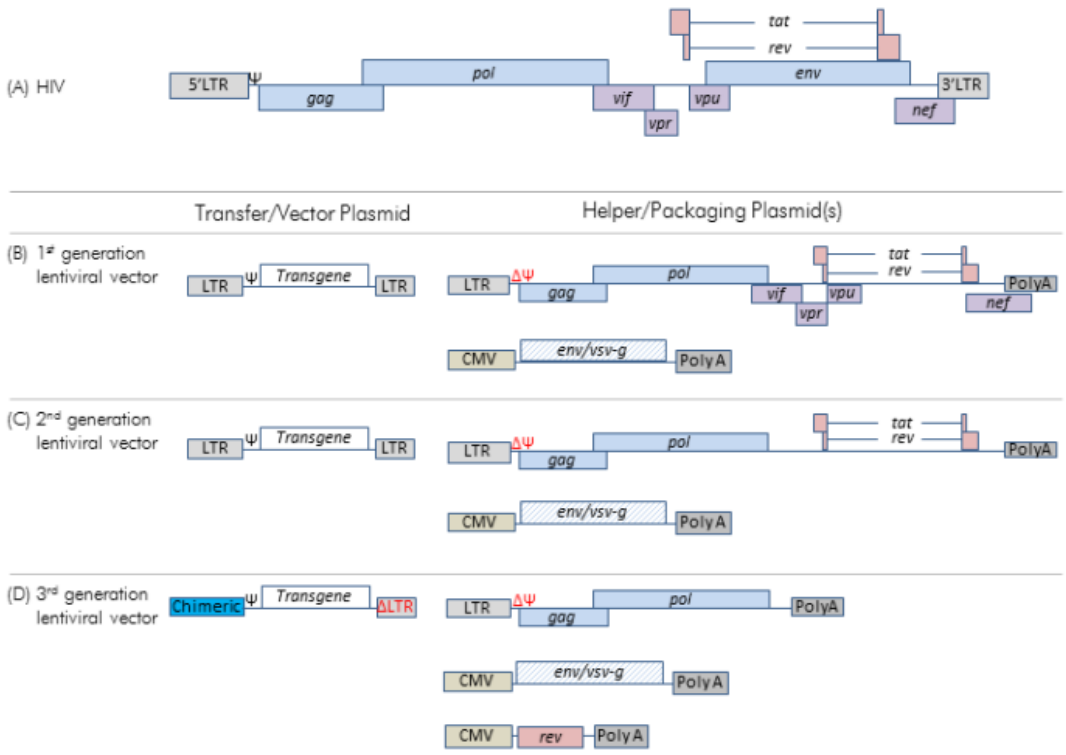

Figure 2-3: Genome of HIV-1 and the evolution to 3rd generation lentiviral vector systems
(A) The HIV genome with common retroviral genes (blue) and HIV-specific genes (purple and pink); note that the figure is not to scale and only meant to show the relative location of genes. (B) A first generation three-plasmid lentiviral vector system. (C) A second generation three-plasmid lentiviral vector system. (D) A third generation four-plasmid lentiviral vector system.

Figure 1 Image was captured from https://www.canada.ca/content/dam/phac-aspc/documents/services/canadian-biosafety-standards-guidelines/guidance/lentiviral-vectors/Lentiviral_Vectors_Guideline_2019.pdf

6. Safety Precautions

6.1 Pathogen and Local Risk Assessment

- According to the Canadian Biosafety Guidelines (PHAC, 2019) lentivirus vectors are generally classified as **Risk Group 2 (RG2)** human pathogens and **Risk Group 2 (RG2)** animal pathogens. However, some characteristics may increase their risk

leading to additional or modified biosafety requirements or to classification as **RG2+/RG3**.

- The appropriate level of risk must be determined by completing and submitting with the Biohazard permit application both a Pathogen and Local Risk Assessment. Decisions about containment should take into account a range of parameters/considerations including:
    a) the nature of the vector system and the potential for regeneration of replication competent virus from the vector components,
    b) the nature of the transgene (e.g., known oncogenes or genes with high oncogenic potential may merit special care)
    c) the vector titer and the total amount of vector,
    d) the inherent biological containment of the animal host, if relevant, • negative RCR testing.
- The table below can be used to assist the PI on determining the appropriate Risk Group for the LVV in use.

Information to Help the Risk Assessment of LVVs		
Pathogen Risk Assessment		
Information to be submitted to the Institutional Biohazard Committee (IBC)	**Low Risk (RG2)**	**High Risk (RG2+/RG3)**
Transgene function	**Non-oncogenic/Nontoxic** Structural proteins Enzymatic proteins Metabolic enzymes Membrane proteins DNA replication, chromosome segregation, mitosis, meiosis; Reporter genes (GFP, luciferase) Growth (cell growth, housekeeping)	**Oncogenic/Toxic** Oncogenes identified by potential of viral and cellular analogs or mutations in tumor suppressors (does not include SV40 T-antigen containing cells) Regulatory genes for transcription and cell activators. (cytokines, lymphokines, tumor suppressors) Cell Cycle, cell division
Number of plasmids used to generate LVVs	3 or 4 or more plasmids	2 or 3 plasmids
Mutations within LVVs	3rd generation transfer plasmid with self-inactivating LTRs and other deleterious mutations.	2nd generation transfer plasmid with wild-type LTRs

	Deletion of viral accessory genes Deletion of *tat* regulatory gene	Expression of viral accessory genes Expression of *tat* regulatory gene
Expression control elements	Weak promoters	Strong promoters (CMV, SV40)
Host range	Non-human tropism Ecotropic, amphotropic	Expanded host range (LVVs pseudotyped with VSV-G)
Concentration	< 1x 10^9 infectious units/mL	> 1x 10^9 infectious units/mL
Manipulation volume	< 100 mL	>100 mL
Percentage of genome deleted or substituted	>2/3	<2/3
Local Risk Assessment		
Animals	Non-permissive hosts (unlikely to shed virions)	Permissive hosts or host engrafted with human cells (more likely to shed virions)
Production volume	Small scale (<10L)	Large scale (>10L)
Manipulations	No use of sharps Unlikely to produce aerosols	Use of sharps May produce aerosols

6.2 Laboratory requirements

The main risk of transmission of LVVs to personnel is through accidental inoculation, cut or punctures with contaminated instruments, and contact with open wounds. Mucous membranes exposure of eye, nose and mouth should be considered when there is a risk of exposure to air droplets contained in aerosolized materials.

Exposure control to LVVs is achieved through the correct application of physical containment requirements, engineering, administrative controls, and personal protective equipment (PPE).

Physical containment requirements and engineering controls

1. Work with LVVs (RG2) must be carried out in a facility that meets containment level 2 physical requirements (CL2) as per Queen's Biosafety Manual (pp 38-39)
    - Lockable doors to the containment lab or zone
    - In case openable windows are present, proper pest control is in place.

- Hand washing sink to be provided close to the entry/exit of the containment zone or laboratory.
- Certified Class II Biological Safety Cabinets
- Vacuum lines equipped with primary and secondary vacuum flask containing a 10% bleach solution.
- Sealed centrifuge rotors and/or safety cups to be used for centrifugation steps.

2. In case additional biosafety requirements are required based on the results of a Pathogen and Local Risk Assessment. (LVVs RG2+/RG3)
    - CL2 physical requirements plus:
        - Dedicated incubator identified by a sign (LVVs RG2+)
        - Directional airflow with negative pressure to the lab.

Administrative Controls

LVVs - CL2

- Controlled access to the containment zone at the discretion of the PI.
- Personnel to be trained in all procedures relevant to the use of LVVs
- Lockable doors to the laboratory or containment zone to remain closed.
- Entry and exit procedures must be developed and posted to the laboratory.
- Safe laboratory working practices apply (shoes with closed toes and no heels are required, long hair tied back, no eating, drinking or smoking, no bare legs, no jewelry, etc.)
- PPE donning and doffing procedure must be developed and posted in the laboratory.
- Work in the BSC will be done according to Standard Operating Procedure: Safe Use of a Class II Biological Safety Cabinet (SOP-Biosafety-03).
- BSC for work with LVVs clearly identified.
- Centrifugation procedures must be developed and posted in the laboratory.
- LVVs storage and transportation procedure must be developed and posted in the laboratory.
- A procedure for testing RCR formation must be implemented and results documented in the laboratory logbook.
- Decontamination and disinfection procedures in place
- A post-exposure response plan should be in place as a component of the medical surveillance plan.
- Emergency response procedure posted in the laboratory.

- **LVVs - CL2+**
    - CL2 admin controls plus:
        - Regular monitoring and control of the containment zone airflow direction.

6.3 Personal Protective Equipment

The following personal protective equipment MUST be worn when working with Lentiviral vectors:

- Gloves (consider double gloving for RG2+). Gloves will be worn during all cell culture manipulation and double gloving will be encouraged since micro-holes may be present in gloves and so that the outer pair can be removed before moving from the biosafety cabinet to prevent the spread of contamination to centrifuges, incubators, and benches.
- Dedicated lab coats or gowns with tight-fitting cuffs will be used to protect any bare skin from contamination.
- Eye safety glasses or goggles (if there is a risk of splashes) face shield (only when working with animals)

6.4 Medical Surveillance (post-exposure prophylaxis)

Before working with lentiviral vectors, a post-exposure response plan (PEP) should be in place as a part of the medical surveillance plan. In case of needlesticks or sharp injuries with first and second generation LVVs, prompt administration of post-exposure prophylaxis within one hour will minimize the already low risk of HIV infection. It is recommended the administration to be done by a health care professional through the Occupational Health Service available for Queen's University employees.

Occupational Health Services for personnel working in and around Queen's research laboratories is available through Walsh and Associates Occupational Health Services. Details and a map are available at https://www.safety.queensu.ca/biosafety/occupational-health-services-biological-hazards. Charges will be billed to departments through the Department of Environmental Health and Safety, and payment is the supervisor's responsibility.

7. Work Practices (LVV special handling procedures)

1. Do not work with the lentivirus or lentivirus-containing materials outside the dedicated room.
2. Plan your work and bring all the materials necessary before the start of the work.
3. Allow enough time to decontaminate the work area.
4. Keep isolated stocks of virus in a second leak-proof container in the -80 C freezer identified for lentivirus storage. Ensure there is a biohazard sign on the freezer and that it is kept locked.
5. It is highly recommended not to use glass, needles, and razor blades for your experimental procedures to reduce the risk of inoculation. Safety engineered sharps and needles that reduce the risk may also be applicable. Plastic transfer pipettes will be used instead of glass Pasteur pipettes to minimize accidental exposure.
6. The lab coat should be removed after completion of work or in the event of a potential/suspected contamination: it must remain in the room and must be disinfected (soaked in bleach 10% for 30 minutes) prior to laundering.
7. A respirator is not needed, but a surgical mask should be worn to protect mucous membranes of the nose and mouth if any manipulation needs to be performed outside of the BSC (e.g., centrifugation). Note that the mask does not provide protection against infectious aerosols.
8. Infectious material will only be taken out of the laboratory if doubly contained in a leak-proof plastic container with a tight-fitting lid to prevent spills, and only if being taken to another facility that has been approved for containment of these agents or for autoclaving or incineration. Virally transduced cells will be fixed, lysed, or extracted using methods that will destroy any lentivirus that might still be present before removal from the containment laboratory.
9. The designated BSC can be used for regular tissue culture work after it has been adequately decontaminated as described in the Standard Operating Procedure: Safe Use of a Class II Biological Safety Cabinet (SOP-Biosafety-03).
10. When possible, an incubator should be reserved for the work and is to be identified by a sign. This is critical for the RG2+category. Keep all virus infected cells in this incubator.
11. Perform all centrifugation in closed containers in sealed cups, using rotors with air-tight sealing caps or centrifugation buckets with air-tight sealing caps. Load and unload these rotors or centrifugation buckets inside the BSC.
12. If the Fluorescent Microscope is kept in a containment level 2 laboratory, transport tissue culture plates to this room, wipe them with 70% ethanol and transfer them to a leak-proof container. Cover them during transportation. After use, decontaminate the surface of the microscope with 70% ethanol.
13. Use a spill tray (e.g. Tupperware with an air-tight fitting lid) to transfer containers of viral material to and from the incubator to contain spills.

14. Incubators should be decontaminated on a regular schedule established by the laboratory supervisor. Approved disinfectants or incubator self-cleaning programs can be used for that purpose.
15. Decontaminate the incubator with 1% sodium hypochlorite (the active ingredient in bleach) according to a regular schedule. This is critical for the RG2+category. Alternative disinfectants such as 1% SDS followed up with 70% ethanol can also be used.
16. Whenever bleach is used on metal surfaces, rinse with 70% ethanol.
17. Media, bacterial and cell cultures will be decontaminated by adding bleach to a final concentration of 10%, followed by a minimal contact time of 30 minutes, prior to disposal down the sewer. Because lentivirus is an enveloped virus, it is susceptible to inactivation by treatment with 70% ethanol. Upon completion of work, the outer surface of everything contained in the biological safety cabinet will be decontaminated with 70% ethanol, before removing all items from the hood. The hood will then be thoroughly washed with 70% ethanol.

7.1 In vivo

- All work performed in vivo with LVVs must be carried out in an animal containment zone (SA or LA) of equal containment level that the assigned RG.
- Additional precautions must be in place when working with HIV permissive animal hosts or those that have been engrafted with human cells.
- Work practices to be developed to minimize the risk of exposure including:
  - Animal restraining procedures
  - When possible, injections to be performed inside a BSC.
  - Where it is not feasible to perform injections in a BSC, additional PPE must be needed to reduce the possibility of mucosal exposure.
  - Disinfecting or cleaning the site of inoculation to eliminate any lentiviral vector that may remain on the surface of the animal.

8. Training requirements

Students and staff must be trained in handling, disposal, and emergency protocols, and must sign in writing that they have understood the training prior to starting the experimental work with the infectious particles. The training must be conducted by a competent individual designated by the Principal Investigator (PI), documented and by the staff /students and by the Supervisor and/or PI, who must also attest that the staff /students have demonstrated proficiency in microbiological practices and techniques.

The laboratory training must address:

- Aseptic techniques and procedures
- Personal protective equipment (e.g., lab coats, goggles, glove selection)
- Signage and labels
- Safe use of centrifugation devices and the ultracentrifuge
- Decontamination and disinfection
- Effective use of biological safety cabinets (BSC)
- Accident and incident reporting procedures
- BSC failure protocols

In addition to the in-house training, personnel must also take the following mandatory safety training courses offered by EHS:

- Biosafety - Level 2 quiz
- WHMIS

9. References

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10. Revision History

Revised	Date	Changes
Queen's Biohazard Committee (IBC)	06-24/2024	**Initial release**