

McGill University

# Standard Operating Procedure (SOP) for Safe Handling of Lentivirus

Environmental Health & Safety

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## Standard Operating Procedure (SOP) for Safe Handling of Lentivirus

**PURPOSE:** This SOP describes the safe handling of lentivirus.

**APPLICATION DATE:**

**APPROVAL DATE:**

**REVISION DATE:**

**PRINCIPAL INVESTIGATOR:**

**DEPARTMENT:**

**BIOHAZARDS PERMIT #:**

**LABORATORY LOCATION (S) FOR LENTIVIRUS HANDLING AND STORAGE:**

**DESCRIPTION OF BIOLOGICAL MATERIAL USED:**

1) Vectors

Packaging vector (e.g. pMDLg/pRRE, pRSV-Rev or psPAX2):

Envelop vector (e.g. pCMV-VSVG or pMD2.G):

Transfer vector (e.g. pLKO or pLX302):

2) Genes:	Function (tumor suppressor, oncogene, etc.)	Modifications (downregulation, overexpression, etc.)

**CONTAINMENT LEVEL:** Level 2 Physical, Level 3 Operational (2+)

**REFERENCE** Canadian Biosafety Standards and Guidelines (CBSG), 1st Edition: Public Health Agency of Canada, 2013

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### 1. INTRODUCTION

The work must be carried out in a facility that meets containment level 2 physical requirements; lab workers must use containment level 3 operational procedures (“containment level 2 with additional precautions”; “containment level 2+”).

For details regarding the containment standards, refer to the Canadian Biosafety Standards and Guidelines (CBSG): <http://canadianbiosafetystandards.collaboration.gc.ca/cbsg-nldcb/index-eng.php?page=1>. The Physical Containment Requirements can be found in Chapter 3 and the Operational Practice Requirements in Chapter 4. If you have any questions, please contact the EHS Biosafety Officer at [ehs@mcgill.ca](mailto:ehs@mcgill.ca).

Laboratory hazards associated with the viral vectors include:

- Penetration through the skin via puncture or absorption (scratches, cuts, abrasions, dermatitis)
- Mucous membrane exposure of eye, nose and mouth

## **2. TRAINING**

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 designated trainer in each lab assigned by the Principal Investigator (PI), documented and signed using Appendix 1 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:

- Introduction to Biosafety
- Safe use of Biological Safety Cabinets
- WHMIS
- Hazardous Waste Management & Disposal Training for Lab Personnel

## **3. SIGNAGE:**

Ensure that signage (Appendix 2) is posted at the entrance of the room and that it includes the following:

- A biohazard warning symbol
- Containment level
- Special provisions for entry, if any
- Emergency contact names and phone numbers
- Visual reminder of key steps to adhere (Appendix 7a & 7b)

## **4. SAFE WORK PRACTICES:**

### **4.1 General**

An "Application to use Biohazardous Materials" must be submitted to EHS for approval prior to the start of the project. Do not start your experimental work until you have received and understood the required training.

- Do not work with the lentivirus or lentivirus-containing materials outside the dedicated room and this room should always remain closed.
- Plan your work and bring all the materials necessary before the start of the work.
- Allow enough time to decontaminate.

- Minimum requirement for packaging system is 2<sup>nd</sup> generation, but 3<sup>rd</sup> generation system is recommended. Only replication-incompetent transfer system can be used. For further packaging information, you may consult this non-profit plasmid repository website: <http://www.addgene.org/lentiviral/packaging/>
- It is highly recommended to not use glass, needles and razor blades for your experimental procedures in order to reduce the risk of inoculation. If you intend to inject the virus into animals such as mice, please contact the Animal Compliance Office before starting your procedures.
- Safe laboratory working practices apply (shoes with closed toes and heels are required, long hair tied back, no eating, drinking or smoking, no bare legs, no jewelry, etc.)
- Wear a lab coat designated for lentiviral work, wear an additional layer of protective clothing (solid front gown) over lab coat if possible. The lab coat and the layer should be removed after completion of work or in the event of a potential/suspected contamination: it must remain in room and must be autoclaved prior to laundering.
- Wear safety glasses and two pairs of clean disposable gloves. The outer gloves are always removed before removing hands from the BSC or in the event of a potential/suspected contamination. Ensure that the gloves cover the end of the lab coat sleeves. Gloves with extra-long wrist protection or disposable arm guard are highly recommended.
- 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.
- If cells are to be transported to another level 2 containment laboratory approved for lentiviral work, a secondary container with an air-tight fitting lid to prevent spills must be used for transport.
- If shoe covers are worn, remove them prior to exiting the laboratory.
- A Class II BSC has been designated for this project, and is identified with a sign. Perform all manipulations with infectious agents in this BSC.
- The designated BSC can be used for regular tissue culture work after it has been adequately decontaminated as described in Section 4.2.3.
- An incubator has been reserved for this project, and is identified by a sign. Keep all virus infected cells in this incubator.
- Use a spill tray (e.g. a Tupperware with an air-tight fitting lid) to transfer containers of viral material to and from the incubator to contain spills.
- Decontaminate the incubator with 1% sodium hypochlorite (the active ingredient in bleach) whenever you finish a new virus preparation or infection. Alternative disinfectants such as 1% SDS followed up 70% ethanol can also be used.
- Whenever bleach is used on metal surfaces, rinse with 70% ethanol
- Always use double bags (e.g ziploc) for contaminated materials inside the BSC, make sure the bags are fully sealed and the exterior decontaminated prior to discarding into the biohazards box for incineration (dry waste only).
- For disposal of liquid waste, refer to section 5.2.

#### 4.1.1 Disinfectants

The two main disinfectants used are 70% ethanol and 1% sodium hypochlorite (the active ingredient in bleach).

70% ethanol is prepared at least once a week and is used for surface disinfection of the BSC and of material entering or leaving the BSC. In case of a spill inside the BSC the contact time should be 20 minutes for cabinet surfaces.

Different brands of bleach might have different concentration of sodium hypochlorite (4-10%). Therefore, please make sure the proper dilution is made to reach 1% sodium hypochlorite final working concentration. Bleach is prepared at least once a week and is used for rinsing tips, pipettes, tubes etc. as well as for medium and cells, as described in Section 5. The contact time should be 20 minutes. In the case of a spill inside the BSC, disinfect all non-metal material for 20 minutes with more concentrated bleach (5% sodium hypochlorite). For a spill outside the BSC disinfect floor, walls, etc. for 20 minutes

with more concentrated bleach (5% sodium hypochlorite). Bleach is effective but volatile and corrosive and cannot be autoclaved due to the release of chlorine gas.

Alternative disinfectants include 1% SDS+70% ethanol, 5% Amphyl (phenolic), 0.5% Wescodyne (iodophor) and other active disinfectants may also be used. For simplicity, this SOP will describe how to use ethanol and bleach for disinfection.

## **4.2 Biological Safety Cabinets:**

Class II BSCs prevent the escape of particulates into the workers' environment and prevent contaminants from the surrounding environment from entering the cabinet.

### **4.2.1 Preparing to work in the BSC**

- Turn the UV light off. Please note that the use of UV light is not recommended due to their limited effectiveness at disinfecting the inside of BSCs.
- Turn on the visible light.
- Switch the cabinet blower on. If the cabinet malfunctions, do not start the experiment, and report it to your supervisor.
- Disinfect surfaces with 70% ethanol.
- Place only the items you will need inside the cabinet.
- Ensure that the front and rear grilles are not blocked by materials.
- Allow the BSC to run for 15 minutes before starting work.

### **4.2.2 Working in the BSC**

- Cover the work surface with absorbent paper (optional).
- Segregate the work area into clean and contaminated sections. Keep contaminated material (discard dry, liquid waste flask) towards the back of the cabinet, do not block the rear grille.
- Place discards in a container lined with double plastic bags inside the BSC.
- Work at least 4-6 inches inside the cabinet window.
- Discard contaminated liquids in a 500 ml bottle containing bleach (final 1% sodium hypochlorite), or other alternative disinfectants, as described in Section 5.2 below
- Keep containers closed when not in use to minimize spills.
- Always remove first pair of gloves before removing hands from the BSC.

### **4.2.3 Preparing to shut down the BSC**

- Leave the blower on for at least 5 minutes to purge the cabinet
- Surface decontaminate all material and equipment with 70% ethanol before removing them from the cabinet. Surface decontamination with 1% sodium hypochlorite and then 70% ethanol is recommended. Surface decontaminate waste bags prior to discarding them into the biohazards boxes.
- Wipe the cabinet surfaces with 70% ethanol.
- Before leaving the lab at the end of the procedure, switch off the visible light
- Close the cabinet sash

### **4.3 Freezer**

- Keep isolated stocks of virus in a second leak-proof container in the -80 C freezer identified for lentivirus storage
- Ensure that there is a biohazard sign on the freezer and that the freezer is kept locked at all times.

### **4.4 Fluorescent Microscope**

Fluorescent Microscope is kept in a containment level 2 laboratory. To transport tissue culture plates to this room, wipe them with 70% ethanol and transfer them to a leak-proof container. Cover them during transportation. In the case of a spill refer to section 6.1.2. After use, surface decontaminate the microscope with 70% ethanol.

### **4.5 Centrifuges**

- Perform all centrifugations 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 in BSC

#### **4.5.1 Ultracentrifuge**

##### **4.5.1.1 Preparing for ultracentrifugation**

- Bring rotor to BSC
- Fill tubes and insert in holders, and screw caps
- Disinfect rotor with 70% ethanol before taking out of the BSC

##### **4.5.1.2 After centrifugation is completed**

- Bring rotor to BSC
- Remove tubes
- Decontaminate the rotor with 70% ethanol
- Return rotor

## **5. WASTE**

### **5.1 Pipettes, tips, tubes, etc.**

- All dry waste should be contained in the double plastic bags (e.g. Ziploc) inside BSC, before discarded into the biohazards box for incineration. Please keep the incineration box closed at all time.
- Decontaminate with bleach (final 1% sodium hypochlorite). Leave for 20 minutes. It is recommended to freshly prepare the diluted bleach prior to starting the experiments.
- Pipettes and tips are considered to be sharps; after decontamination, discard them in the biohazards box.
- Decontaminate all non-sharp material (tubes, flasks, etc.) with bleach or other disinfectant, then discard in the biohazards box.

## 5.2 Liquid medium and solutions:

- Pipette the liquid waste into a 500 ml bottle containing bleach (with final 1% sodium hypochlorite in this 500 ml total volume). For example, add 50 ml of bleach that contains 10% sodium hypochlorite in this 500 ml bottle. Alternative disinfectants described earlier can also be used.
- In order to prevent aerosol, vacuum system is prohibited to be used to remove lentivirus-containing liquid.
- Leave for 20-30 minutes
- Pour disinfected liquids down the sink and let cold water run for minimum 5 minutes. The bottle can be rinsed and reused. Do not autoclave liquids disinfected with bleach.
- If 1% SDS is used to decontaminate the liquid waste, additional autoclaving step can be used further ensure the complete inactivation of lentivirus.

## 6. EMERGENCY PROCEDURES:

Report all spills, exposures and accidents, no matter how minor, to the lab supervisor and to Environmental Health and Safety. In the event of a serious spill, contact McGill Emergency at local 3000. *Accident/Incident/Occupational Disease* and *Spill Report* forms are attached in Appendices 3 and 4. Complete the report form and forward it to Environmental Health & Safety (EHS) at [ehs@mcgill.ca](mailto:ehs@mcgill.ca)

### 6.1. Spills

The procedures to follow in case of a spill should be posted on the wall beside the biosafety cabinet (Appendix 5).

#### 6.1.1 Spillage inside BSC:

- Leave cabinet running for 10 minutes to allow aerosols to be HEPA filtered before cleaning the spill.
- Before removing your hands from the BSC, remove contaminated or potentially contaminated gloves (outer set) and place them into the double plastic bags inside the BSC, close and place in biohazards box for incineration.
- Remove inner pair of gloves and discard them in the biohazards box.
- If the laboratory coat is contaminated, put it inside a biohazards bag for autoclaving.
- Wash hands thoroughly with soap. Put on new gloves, lab coat and/or solid front gown.
- Place absorbent papers presoaked with disinfectants (bleach solution with 1% sodium hypochlorite or 1% SDS) over the spill area. Pour 70% ethanol on the absorbent paper, starting at the perimeter and working towards the center of the spill. Let sit for 20 minutes
- If spilled material goes through the work surface into the catch tray, pour 70% ethanol into the catch tray and let sit for 20-30 minutes. Lift the work surface and use long tongs to absorb the spill with paper towels and transfer the absorbent paper to the double plastic bags, close and place in biohazards box for incineration.
- Decontaminate all non-stainless steel surfaces of materials and equipment inside the BSC with concentrated bleach (5% sodium hypochlorite) solution (or other effective disinfectants) for 10 minutes prior to removal from cabinet. Rinse with water.
- Decontaminate all interior surfaces of the BSC with 70% ethanol or other disinfectant. Let sit for 20 minutes. Rinse with distilled water. Surface decontaminate all spill cleanup materials with concentrated bleach (5% sodium hypochlorite) for 10 minutes
- Remove gloves and gown.

### 6.1.2 Spillage outside BSC:

- As for the above procedure but using concentrated bleach (minimum 5% sodium hypochlorite) for 20-30 minutes. Rinse with water. Do not forget to decontaminate all areas including walls, cabinets, floor in proximity of the spill.

### 6.1.3 Spill inside a centrifuge (ultracentrifuge, microfuge):

If a tube breaks during centrifugation, liquids will be confined to the inside of the rotor. The decontamination procedures to follow are:

- Handle the contaminated rotor inside the BSC.
- Transfer the spilled liquid from the rotor into a 500 ml bottle containing 50 ml of 10% sodium hypochlorite.
- Using forceps, remove tubes and tube fragments and decontaminate them with a solution of 5% sodium hypochlorite solution for 10 minutes. Discard as described in Section 5.1
- Soak the rotor in 70% ethanol for 20 minutes. Wash with a mild detergent, rinse with water and allow to dry.
- Wipe down the inside of the centrifuge with 70% ethanol.

## 6.2 BSC failure

- Cap cultures, surface decontaminate with 70% ethanol and return them to the incubator.
- Surface decontaminate all materials inside BSC with 70% ethanol before removing them.
- Remove waste and place in biohazards box for incineration.
- Close the sash and turn off the blower motor switch.
- If the failure is caused by a power outage, restart and decontaminate the BSC as described in Section 4.2.1 when the power returns.
- If the failure is due to BSC malfunction, decontaminate cabinet surfaces with 70% ethanol and contact H.E.P.A. Filter Services (514-871-9990) or another company's coordinates for repair. Ensure that the service rep decontaminates the cabinet with formaldehyde before carrying out the repair.
- Affix a warning sign (e.g., "OUT OF ORDER. DO NOT USE") to the cabinet.

## 7. EXIT PROTOCOLS:

Before leaving the facility:

- Remove gloves and dedicated lab coat. The lab coat must not leave the room where the lentivirus work is performed except for autoclaving; hang it inside the facility before leaving.
- Prior to autoclaving place the dedicated lab coat in a biohazard bag.
- Wash your hands.

Note: You do not have to remove your lab coat if you are not working on this project.

## 8. APPENDICES

- Appendix 1 Training sign-off sheet, supervisor's proficiency attestation
- Appendix 2 Signage
- Appendix 3 Accident/Incident/Occupational Disease Report form
- Appendix 4 Spill Report Form
- Appendix 5 Emergency Procedure for Spill inside BSCs
- Appendix 6 Estimation of remaining virus
- Appendix 7(a&b) Visual reminder of key steps



## Appendix 1: Documentation of Training

Trainer: \_\_\_\_\_  
Print name

Trainee: \_\_\_\_\_  
Print name

Training Modules	Trainer's Signature	Trainee's Signature	Date (DD/MM/YY)
<input type="checkbox"/> PHAC Guidelines			
<input type="checkbox"/> Signage and labels			
<input type="checkbox"/> Entry and exit procedures			
<input type="checkbox"/> Personal protective equipment (lab coats/gowns, gloves, eye protection, etc.)			
<input type="checkbox"/> Aseptic techniques and procedures			
<input type="checkbox"/> Safe use of biological safety cabinets			
<input type="checkbox"/> Safe use of centrifuges and other equipment			
<input type="checkbox"/> Decontamination and disinfection			
<input type="checkbox"/> Waste handling and disposal			
<input type="checkbox"/> Spill response and other emergency protocols			
<input type="checkbox"/> Accident and incident reporting procedures			

### Principal Investigator's Statement:

The above trainee has demonstrated proficiency in the practices and techniques required for work on this project.

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

Appendix 2: Signage

<p><b>AUTHORIZED PERSONNEL ONLY</b></p> <hr/> <p>THIS CONTAINMENT ZONE CONTAINS INFECTIOUS MATERIALS AND/OR TOXINS</p>

<p><b>BIOHAZARD</b></p>
<p>CONTAINMENT LEVEL: <u>2</u></p> <p>PRIMARY CONTACT PERSON: _____</p> <p>PHONE NUMBER: _____</p> <p>ALTERNATE EMERGENCY CONTACT: _____</p> <p>PHONE NUMBER: _____</p> <p>ENTRY REQUIREMENTS: _____</p> <p>_____</p> <p>_____</p> <p>_____</p>

**Appendix 3:** [Accident/Incident/Occupational Disease Report](#) form

**Appendix 4:** [Spill Report Form](#)

## Appendix 5: Spillage inside BSC

- Leave cabinet running for 10 minutes
- Remove contaminated or potentially contaminated gloves (outer set) and place them into the double plastic bags inside the BSC, close and place in biohazards box.
- Remove inner pair of gloves and discard them in the biohazards box.
- If the laboratory coat is contaminated, put it inside a biohazards bag for autoclaving.
- Wash hands thoroughly with soap.
- Put on new gloves, lab coat and/or solid front gown.
- Place absorbent papers presoaked with disinfectants (bleach solution with 1% sodium hypochlorite or 1% SDS) over the spill area.
- Pour 70% ethanol on the absorbent paper, starting at the perimeter and working towards the center of the spill. Let sit for 20 minutes
- If spilled material goes through the work surface into the catch tray, pour 70% ethanol into the catch tray and let sit for 20-30 minutes. Lift the work surface and use long tongs to absorb the spill with paper towels and transfer the absorbent paper to the double plastic bags, close and place in biohazards box.
- Decontaminate all non-stainless steel surfaces of materials and equipment inside the BSC with concentrated bleach (5% sodium hypochlorite) solution for 10 minutes prior to removal from cabinet.
- Rinse with water.
- Decontaminate all interior surfaces of the BSC with 70% ethanol or other disinfectant. Let sit for 20 minutes. Rinse with distilled water.
- Surface decontaminate all spill cleanup materials with concentrated bleach (5% sodium hypochlorite) for 10 minutes
- Remove gloves and gown.

## Appendix 6: Estimation of remaining virus

To calculate the reduction in free virus particles use the following formula:

$$(20^W * 200^I * 2^{2.4T}) / V = >100^*$$

\*The reduction factor must be at least 100.

Example:

W	= wash steps	W= 2
I	= inactivating steps	I = 1
T	= days culturing	T = 4
V	= starting virus titer	V = $1 \times 10^6$

$$(20^4 * 200^2 * 2^{9.6}) / 1 \times 10^6 = 62$$

At this point, the reduction factor is 62x which is less than 100x, therefore, CL2+ practices must still be followed.

However, when W= 2, I=2, T=2 and V= $1 \times 10^6$ , then the answer will be  $(20^2 * 200^2 * 2^{4.8}) / 1 \times 10^6 = 446$

Now the original titer of  $1 \times 10^6$  has been reduced with a factor 446 (more than 100x reduction factor) generating a stable cell line which can be safely handled in regular CL2 physical and operational settings.

Appendix 7a: Visual reminder of key steps

# PERSONAL PROTECTIVE EQUIPMENT

DOUBLE GLOVES

WRISTS COVERED

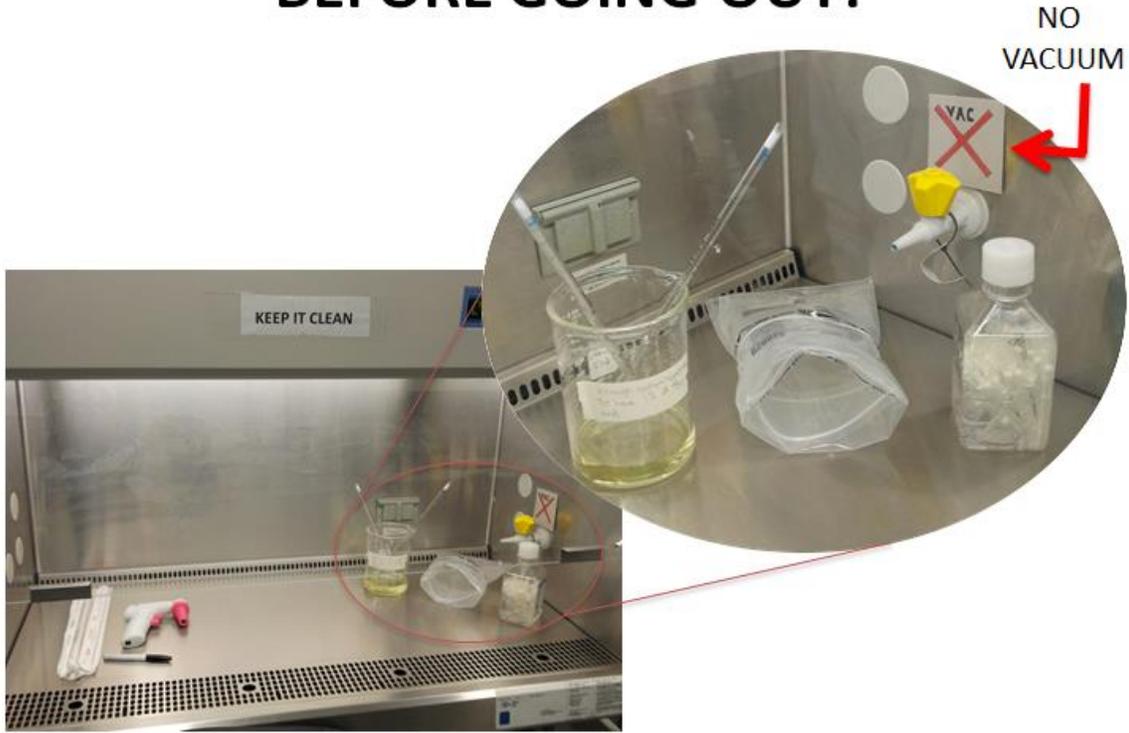
SAFETY GLASSES



LONG PANTS  
&  
CLOSED SHOES

Appendix 7b: Visual reminder of key steps

# ENSURE INACTIVATION OF VIRUS BEFORE GOING OUT!



That is....



how to transport infected cultures