Standard Operating Procedure for Safe Handling of Lentivirus

Environmental Health & Safety

Responsible person:	ULSC Committee	Date of Issue:	November 13th, 2020
Written by:	Ruth Blanchette	Issued By:	ULSC
Document control number:	EHS-BIOS-200	Revision #:	2.0
		Revision Date:	November 11th, 2020
Pages:	12	Revised By:	Ruth Blanchette

I. SCOPE

APPLICATION DATE:
APPROVAL DATE:
REVISION DATE:
PRINCIPAL INVESTIGATOR:
DEPARTMENT:
BIOHAZARDS PERMIT #:

II. PURPOSE

This SOP describes the safe handling of first and second generation lentivirus. Work with Lentivrus is done under the authority of the Public Health Agency of Canada's Human Pathogens and Toxins Licence issued to McGill University. 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, 2nd edition: https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/second-edition.html. 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.

III. HAZARDS

Reference: Canadian Biosafety Standard, 2nd edition: Public Health Agency of Canada

Laboratory hazards associated with viral vectors include:

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

IV. RESPONSIBILITIES

Principal Investigator (PI)

- Ensures the personnel under his/her supervision are trained on the safe and proper handling of Lentiviral vectors
- Ensures this SOP is followed

Designated Person (is a member of the lab designated by the PI to oversee the implementation of this SOP)

- Oversees the implementation of this SOP
- Provides “in-house” training to personnel in the safe handling of Lentiviral vectors

Trained personnel (can include McGill staff, students or others who are trained to handle Lentivirus in the lab)

- Must receive proper training from their PI or designated person on the safe handling of lentivirus.

Note: that after the training takes place, the training must be documented in *Annex 1 – Documentation of Training for SOP – EHS-BIOS-200 - Standard Operating Procedure (SOP) for Safe Handling of Lentivirus*

- Must complete training in the Safe Use of Biological Safety Cabinets (EHS-BIOS-201)
- Shall report any injuries, accidents or spills to their PI and to Environmental Health and Safety via the McGill Accident, Incident & Occupational Disease Report Form.

Environmental Health and Safety – McGill University (EHS)

- EHS maintains this SOP
- Provides and maintains the training courses “Introduction to Biosafety”, “Safe Use of Biological Safety Cabinets”, “WHMIS 2015”
- Provides access to the “Hazardous Waste Management and Disposal” training
- Maintains the records of PIs handling lentivirus under the authority of McGill’s HPTA Licence
- Monitors compliance with this SOP

V. SAFE WORK PRACTICES

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 the work area.
- Minimum requirement for packaging system is 2nd generation, but 3rd 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 no heels are required, long hair tied back, no eating, drinking or smoking, no bare legs, no jewellery, 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 containment level 2 laboratory approved for lentiviral work (CL2+), 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.
- Identify a Class II BSC which 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 the Standard Operating Procedure: Safe Use of a Class II Biological Safety Cabinet (EHS-BIOS-201)..
- An incubator should be reserved for this project, and is to be 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 with 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 V.2.

2. 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 V. 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. If another disinfectant is used it must be specified in the laboratories "Application to Use Biohazardous Materials".

3. Biological Safety Cabinets:

Work in the BSC is to be done in accordance with Standard Operating Procedure: Safe Use of a Class II Biological Safety Cabinet (EHS-BIOS-201).

4. 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.

5. Fluorescent Microscope

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. In the case of a spill refer to section 6.1.2. After use, surface decontaminate the microscope with 70% ethanol.

6. 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

6.1. Preparing for centrifugation/ultracentrifugation

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

6.2. After centrifugation/ultracentrifugation is completed

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

V. 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 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

VI. RELATED DOCUMENTS

SOP EHS-BIOS-201 – Safe Use of Biological Safety Cabinets

VII. 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

VII. REVISION HISTORY

Version	Modification	Biosafety Officer	ULSC Chair	Date
1.0	Initial SOP	Elizabeth St.Louis		2013
1.1	Create fillable PDF form	Ruth Blanchette	Alvin Shrier	15-Apr-2014
2.0	1. Update to Biosafety SOP template 2. Reference new SOPs and Biosafety documents 3. Define responsibilities	Ruth Blanchette	Alvin Shrier	11-Nov-2020

McGill

Appendix 1: Documentation of Training

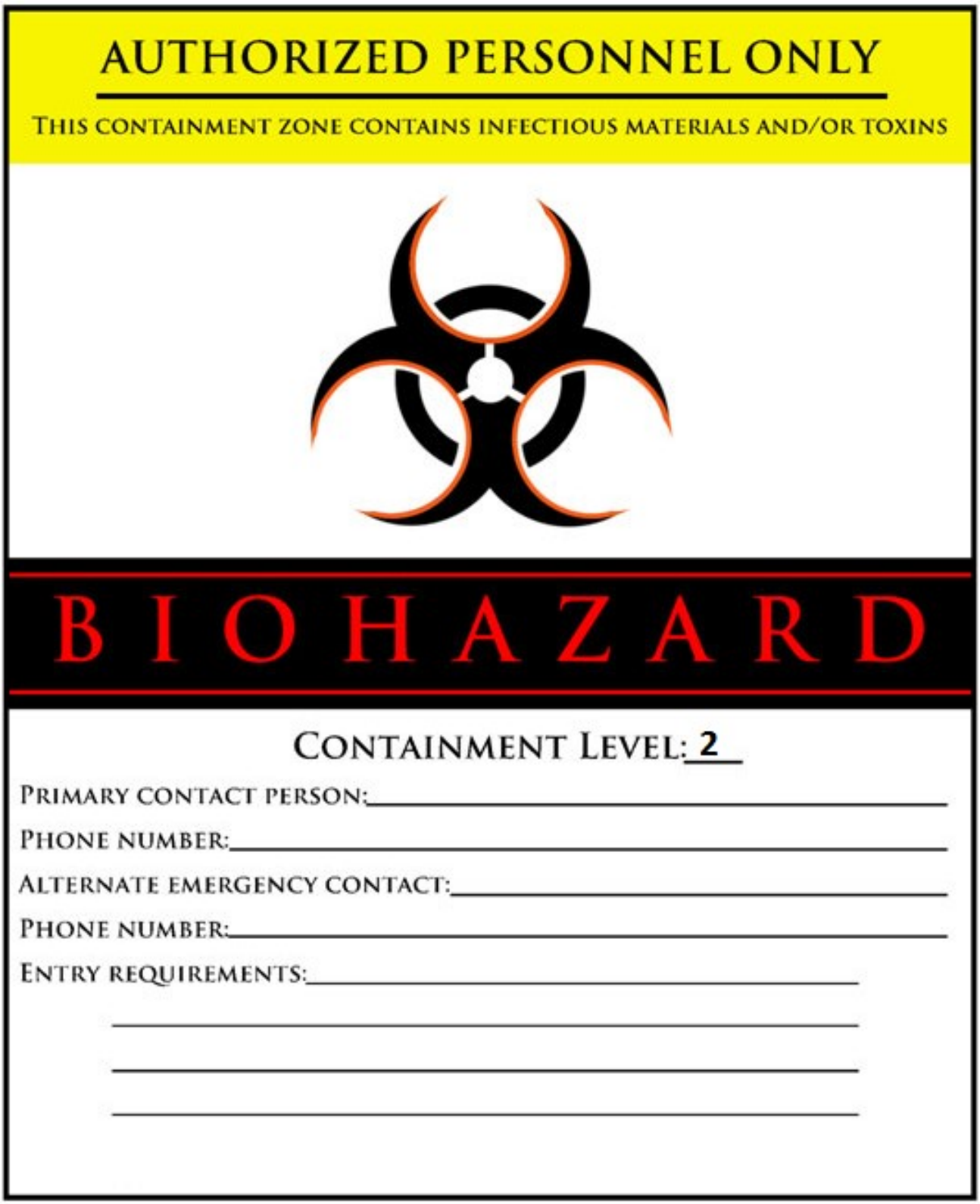
Trainer: _____ **Trainee:** _____

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



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 = 1x10^6

$(20^{4} * 200^{2} * 2^{9.6}) / 1x10^6 = 62$

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=1x10^6, than the answer will be $(20^{2} * 200^{2} * 2^{4.8}) / 1x10^6 =$ 446

Now the original titer of 1x10^6 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

Appendix 7b: Visual reminder of key steps