

**CONTAINMENT LEVEL 2 WITH LEVEL 3 OPERATIONAL PROCEDURES:**

**A GUIDELINE FOR LENTIVIRAL VECTORS AND OTHER CL2+ AGENTS**

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Name, Principal Investigator & Permit Holder Date

**SIGNATURE PAGE**

All the lab members **MUST** read this manual before beginning work with lentiviral vectors. The Principal Investigator should update this manual with site-specific information. Please acknowledge that you have read this manual by printing and signing your name below.

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1. INTRODUCTION:

1.1 Containment Levels

The containment level (CL) provides the end-user with a description of the minimum physical containment requirements, operational practice requirements, and performance and verification testing requirements for handling pathogens safely in a laboratory setting. CL range from a basic laboratory CL-1 to CL-4, the highest level of containment. Refer to the [Canadian Biosafety Standard, 2nd Ed.](https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/second-edition.html) for full descriptions of the containment levels. A complete risk assessment and review by the biosafety committee will determine the appropriate containment level for the specific research.

Previous risk assessments have determined that safety practices over and above those required at CL-2 are generally needed for the safe handling of lentiviral vectors, yet a more complex physical design of the CL-3 laboratory is not necessary. CL-2 + is a term frequently used to describe laboratories where work with microorganisms is conducted in a laboratory meeting the facility structure requirements for Containment Level 2 using biosafety practices, procedures and equipment typically found in CL-3.

The infectious agents used in the CL-2+ facility are generally transmissible following ingestion, exposure to mucous membranes, or intradermal exposure. Regulatory agencies do not provide a standardized list of microorganisms, viral vectors, or research projects that should be conducted at CL2+. Instead, Canadian regulatory agencies describe additional (generally CL3 procedures) for handling specific microorganisms or human specimens expected to contain them in [agent-specific directives and advisories](https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/biosafety-directives-advisories-notifications.html).

See [Appendix B](#AppendixB) for some examples of Risk Group -2 agents that require CL-3 operating procedures under certain conditions. The most commonly used viral vector system that requires CL-2+ containment are lentiviral vectors. The intent of this manual is to provide a standardized guideline for all researchers working with Lentivirus; however, it may be used as a guideline to work with any other biological agent requiring CL-2+ containment based on the risk assessment and incorporation of agent-specific considerations.

**1.2 Lentiviral Vectors**

Lentiviral vector systems are derived from viruses belonging to the *Retroviridae* family, a family of single-stranded, spherical, ribonucleic acid (RNA) viruses. Lentiviral vectors are most commonly derived from human immunodeficiency virus type 1 (HIV-1). However, they may also be derived from HIV-2 and non-human lentiviruses including simian immunodeficiency virus (SIV), feline immunodeficiency virus (FIV), equine infectious anemia virus (EIAV), and bovine immunodeficiency virus (BIV). Lentiviral vectors stably insert a transgene into the genome of a target cell. These vectors have been engineered to infect the target cell but not able to replicate following the infection. However, since most lentiviral vectors are HIV-derived, concerns relating to the potential for the vector reverting to an infectious state remain. The transgene also poses risks, including known and unknown on-target and off-target effects.

*1.2.1 Replication-Deficient Lentiviral Vectors*

To overcome the risk of accidental infection certain lentiviral vectors are designed to be less pathogenic than wild-type lentiviruses due in part to the separation of genes required for packaging of viral particles onto several plasmids, replacement of the native lentiviral envelope protein, and elimination of accessory genes that are essential for replication of wild-type lentiviruses. Lentiviral vector systems designed with these enhanced safety features are not able to replicate in human cells and are defined as replication-deficient lentiviral vectors. 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.

Lentiviral vectors are commonly used in gene transfer applications because of their ability to infect both dividing and non-dividing cells, and hard-to-transfect primary cells. They are designed based on the Human Immunodeficiency Virus (HIV)-1. Refer to the [HIV Pathogen Safety Data Sheet](https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/human-immunodeficiency-virus.html) for an overview of the risks associated with the wild-type virus.

Primary hazards associated with lentivirus work are:

1. Potential for a generation of replication-competent lentivirus (RCL) if exposure occurs during the step where the virus is reassembled in the packaging cell line
2. Potential for insertional mutagenesis and deletions/insertions in the event of a personal exposure at any time after the virus has left cells and is in the supernatant (e.g. an insert that codes for a toxin, activates oncogene or inactivates tumor suppressor genes)
3. Vector titer and volume (as these increase, so does the risk of exposure).
4. The ability of the vector to transduce a wide range of cells.
5. Potential for seroconversion, even with a non-replicating virus, leading to a false-positive HIV test result.
6. Failing to understand that although safety-engineered and commercially available lentivirus vectors are Risk Group 2 agents, work with them still requires compliance with CL2+ facility design or operational practices outlined in this guidance document.

For information on different generations of lentivirus, see [Appendix A](#AppendixA).

2. FACILITY REQUIREMENTS

2.1 Room

1. Dedicated room/rooms.
2. Controlled access to the room.
3. A log of entry & exit – in hardcopy if electronic access logs are not available.
4. Negative pressure.
5. Anteroom: The area designated for donning and removing isolator suits.
6. A sink.
7. Washable floors, walls, counters, and if possible ceilings.

2.2 Equipment

1. Biosafety Cabinets – Class II (NSF accredited and certified).
2. Centrifuge with bio-safety centrifuge rotors.
3. Ultra-Centrifuge with bio-safety centrifuge rotors.
4. CO2 incubator.
5. Balance.
6. Water bath.
7. Dedicated pipette.
8. Fridge and Freezer.
9. Autoclave.
10. Microscope.

2.3 Supplies

1. Filtered Pipette Tips.
2. Set of Pipettes.
3. Kim wipes.
4. Gloves (all sizes) – longer cuffs recommended.
5. 1.5ml, 15ml, 50ml Tubes; plastic disposable serological pipets; and anything else needed for an individual experiment.
6. Bleach – Sodium Hypochlorite tablets
7. Sodium dodecyl sulfate (SDS)
8. Ethanol
9. Isolator Gowns

3. AGENTS USED, LOCATIONS, AND STORAGE

**3.1 Agents used**

Fill in here.

**3.2 Location of use**

Fill in here.

**3.3 Location of storage**

Fill in here.

4. PERSONAL PROTECTIVE EQUIPMENT AND ENTRY-EXIT PROCEDURE

4.1 Personal Protective Equipment Requirements

1. Lab Gown (must reach well beyond the knee)
2. Lab Coat
3. Gloves (recommended longer cuff – 12inch)
4. Full covering shoes
5. Full-length pants
6. Long hair tied back
7. Safety Goggles (even if you wear glasses) – only needed when working with lentivirus outside of the BSC.

Refer to [Appendix C](#AppendixC) for illustration

**4.2 Personal Protective Equipment Procedures**

4.2.1 Entering the Virus Room

1. Tie back hair and loose clothing.
2. Put on a lab coat over a base of long pants and a shirt.
3. Thoroughly wash hands.
4. All open wounds, cuts, scratches, and grazes must be covered with water-proof bandages prior to entry into the room.
5. If a full anteroom is available, put on the reusable lab gown there. If there is no ante-room, enter the room and immediately put on the lab gown. (The lab gown should be labeled and hung next to the door for easy access).
6. Once the lab gown is on, put on the first layer of examination gloves. This is easiest with longer cuffed gloves. Tape to gown cuffs.
7. Don the second pair of gloves in a color different from the first. These should be shorter than the first set for easier top glove changes.

***Note***: No item brought into the room may leave the room unless decontaminated with 70% ethanol. This means all papers and protocols brought into the room may not leave the room unless disinfected first. Disinfection can be done by bagging and autoclaving. You may also write data using pencil then soaking the paper in ethanol. However, this is more problematic and less effective.

4.2.2 Exiting the Virus Room

1. When finished work, ensure that all waste is disposed of as per Section 6.
2. Spray outer gloves with 70% ethanol. Then remove them and place them in the biohazard bag.
3. Before exiting the room, remove the lab gown and hang on the provided hangers. If the gown is contaminated, discard it into the biohazard waste container.
4. If work is complete, discard inner gloves in the biohazard waste.
5. Exit the room, completing the sign out procedure if done for the session.
6. If moving samples to a microscope or storage outside of the room, then inner gloves and glasses must remain on.

***Note:*** It is recommended to use a cart to move samples from one location to another. Remember to remove one glove for opening and closing doors.

5. GENERAL SAFE WORK PRACTICES

1. Record your entry and exit of the room.
2. Do not work with the lentivirus or lentivirus-containing materials outside the dedicated room
3. Plan your work and bring all the materials necessary before the starting work.
4. Allow enough time to decontaminate work surfaces and materials.
5. It is highly recommended to avoid using 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 refer to *BIO-SOP-009 Recombinant Viral Work in Animal Facility* before starting your procedures.
6. Practice safe laboratory work practices (shoes with closed toes and heels are required, long hair tied back, no eating, drinking or smoking, no bare legs, no jewelry, etc.)
7. Wear a lab coat designated for the work and put an additional layer of protective clothing (solid front gown) over the lab coat. The lab coat and gown will be removed after completion of work or in the event of a potential/suspected contamination: they must remain in the room and must be autoclaved prior to laundering.
8. 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. It is helpful to have longer inner gloves covering cuff and shorter outer glove for easy removal. Gloves with extra-long wrist protection or disposable arm guard are highly recommended.
9. A respirator is not required ( however, this can vary based on risk assessment), but a surgical mask or face shield should be worn to protect mucous membranes of the nose and mouth from splashing if any manipulation needs to be performed outside of the BSC (e.g. centrifugation). Note that the mask/face shield does not provide protection against infectious aerosols.
10. If cells are to be transported to another level 2 containment laboratory approved for lentiviral work, a secondary container with leak-proof lid ( with sufficient absorbent materials) must be used for transport to prevent spills.
11. If shoe covers are worn, remove them prior to exiting the laboratory.
12. Designate a Class II BSC for this project and is identified with a sign. Perform all manipulations with infectious agents in this BSC.
13. The designated BSC can be used for regular tissue culture work after it has been adequately decontaminated as described in Section 6.
14. A designated incubator is used for the project and is identified by a sign. Keep all virus-infected cells in this incubator.
15. Use a spill tray (e.g. a sealed, leak-proof, labeled container) to transfer containers of viral material to and from the incubator to contain spills.
16. Decontaminate the incubator with 1% sodium hypochlorite (the active ingredient in bleach) whenever you finish a new virus preparation or infection. Rinse well with sterile distilled water and finish with a final wipe of 70% ethanol. Alternately, 1% SDS followed by 70% ethanol can also be used. Removable shelves and brackets could be autoclaved.
17. Whenever bleach is used on metal surfaces, rinse with 70% ethanol
18. 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).
19. For disposal of liquid waste, refer to the waste disposal section 6.

5.1 Working with Lentivirus outside of Virus Room

1. If the lentivirus will be transported outside the virus room, then a Lab Coat must be worn under the lab gown.
2. Remove the lab gown as mentioned in 4.2.2. Keep the inner gloves, glasses and lab coat on.
3. Place samples (which are within a sealed, leak-proof, labeled container) on a cart and walk carefully to the equipment needed. Note that one glove should be removed for opening and closing doors.
4. Carry a second pair of gloves and put them on before removing the samples from a sealed, leak-proof, labeled container.
5. When working with lentivirus in the room(s), the room must be isolated from general use. Place a sign on the door and keep the door closed.
6. When finished working with the virus, clean the equipment as per section 6.2. Spray the outer pair of gloves with the 70% ethanol and discard them in the biohazard waste.
7. Return the lentiviral samples to the virus room. Note that sections 4.2.1 and 4.2.2 will again need to be followed.

6. ROUTINE PROCEDURES

6.1 Biosafety Cabinet Procedures

1. The BSC blowers need to be operated at least 15 minutes before beginning work to allow BSC to purge. This will remove any particulates in the cabinet.
2. The work surface, the interior walls (not including the supply filter diffuser), and the interior surface of the sash should be wiped with 70% ethanol. Similarly, the surfaces of all materials and containers placed into the cabinet should be wiped with 70% ethanol to reduce the introduction of contaminants to the cabinet environment.
3. Place all necessary materials in the BSC before beginning work. This will serve to minimize the number of arm movements across the fragile air barrier of the cabinet. The rapid movement of a worker’s arms in a sweeping motion into and out of the cabinet will disrupt the air curtain and may compromise the partial barrier containment provided by the BSC. Moving arms in and out slowly, perpendicular to the face opening of the BSC will reduce this risk. Other personnel activities in the room (e.g., rapid movement, opening/closing room doors, etc.) may also disrupt the cabinet air barrier. For this reason, access to the work area must be restricted when work is in progress.
4. Before beginning work, the researcher should adjust the stool height so that his/her face is above the front opening such that they are looking through the glass sash.
5. Spray gloved hands with 70% ethanol before placing them in the cabinet. Manipulation of materials should be delayed for approximately 1 minute after placing the hands/arms inside the BSC. This allows the cabinet to stabilize and to “air sweep” the hands and arms to remove surface microbial contaminants.

Note: When the user’s arms rest flatly across the front grille, room air may flow directly into the work area, rather than being drawn through the front grille. Raising the arms slightly will alleviate this problem. The front grille must not be blocked with research notes, discarded plastic wrappers, pipetting devices, etc. All operations should be performed on the work surface at least 4 inches from the inside edge of the front grille.

1. Equipment that causes turbulence (centrifuge, vortex, etc.) should be placed in the back 1/3 of the work surface. All other work in the cabinet must stop while the apparatus is running.
2. Separate clean and contaminated items. Minimize the movement of contaminated items over clean items (work from clean to dirty).
3. All items used must be decontaminated before leaving the BSC. See Section 6.2 for further information.
4. When work has been finished and all items decontaminated, remove everything from the cabinet and spray the work surface, the interior walls (not including the supply filter diffuser), and the interior surface of the sash with 70% ethanol.
5. The BSC blowers need to run at least 15 minutes to purge any particulates in the cabinet. After 15 minutes, the cabinet may be turned off if you are the last one to use the cabinet for the day. Otherwise, leave the cabinet running for the next person.
6. Ensure that the sash is closed and the cabinet turned off at the end of the day.

6.2 Decontamination of Supplies and Equipment

Lentiviral vectors are labile, so surfaces can be easily decontaminated by 10% bleach and 70% ethanol, with sufficient contact time. The disinfectants are prepared at least once a week and labeled with the date and concentration. Solutions older than 1 week must be discarded and fresh solutions made up for use. Commercial preparation of bleach comes in different concentrations of active ingredient sodium hypochlorite. Please make sure that the final working concentration is 1% sodium hypochlorite.

If stock bottles are kept for more than 1 month after opening, test with chlorine test strips prior to use to verify it is still active and has the right strength. Otherwise, you may need to increase working solution concentrations.

Waste items exposed to bleach must be stored 48 hours to allow for complete deactivation of the sodium hypochlorite. Bag these items separately and labeled with the earliest safe autoclave date.

If the facility or room contains an autoclave then all contaminated waste can be stored in appropriate bags, and autoclaved for sterilization. Once the items have been autoclaved, they may be disposed of as per the building's guidelines.

6.2.1 Small Disposable Plasticware

1. Place a beaker with 10% bleach (for one-time use) or a bottle + cap with 10% bleach (maximum service period of 48 hrs) in the BSC. Be sure to spray the outside with 70% ethanol.
2. All tips and other small items must be treated in this beaker or bottle before being disposed of. All surfaces must be covered by the disinfectant.
3. All items must be treated for at least 30 minutes.
4. The items may then be disposed of as per your building’s waste disposal procedures.\*
5. If you are using a re-usable bottle to bleach the items, be sure to cap the bottle and spray the outside with 70% ethanol. The bottle may then be stored outside of the cabinet, but within the virus lab for up to 2 days.

\*Remember if bleach is used these materials cannot be autoclaved for 48 hours, as they will cause an explosion.

6.2.2 Serological Pipettes

1. Place a pipette bucket or washer next to the BSC and fill with 10% bleach.
2. Treat the ends of the serological pipette with 10% bleach for 10 minutes in the BSC using the beaker mentioned in 6.2.1.
3. Then remove the pipettes and place them in the pipette bucket next to the cabinet. Be sure to remove the gauze from re-usable pipettes before placing them in the bucket.
4. The pipettes must be treated for at least 30 minutes.
5. Disposable pipettes must be disposed of in a cardboard box lined with a biohazard bag and marked.\*
6. Glass pipettes must be placed in an autoclavable bag. The bag must then be autoclaved before the pipettes may be used again.

\*Follow the disposal procedures for the building. Remember if bleach is used these materials cannot be autoclaved for 48 hours, as they will cause an explosion.

6.2.3 Culture Plates

1. Fill each well with 10% bleach.
2. The plates must be treated for at least 30 minutes.
3. The liquid is then removed and the plates can be disposed of following the disposal procedure for the building.\*

\* Remember if bleach is used these materials cannot be autoclaved for 48 hours, as they will cause an explosion.

6.2.4 Liquid Waste

1. For a 1L vacuum flask, place 100ml of concentrated bleach in the empty flask.
2. Then attach the flask to the vacuum line within the BSC. Note that it is required that a 0.2µm in-line filter be installed on the exhaust line of the vacuum system.
3. Liquid waste is then aspirated into a flask using the vacuum line in the BSC.
4. When work is finished, bleach is aspirated through the vacuum line to decontaminate it.
5. The liquid waste must be treated with bleach for at least 30 minutes.
6. When the flask is full or it is the end of the day, the mixture is neutralized with sodium bisulfite and carefully poured down the sink to avoid splashing. Additional water is flushed down the drain, rinsed with copious amounts of water.
7. Then refill the flask with 100ml of bleach and return to the BSC.

6.2.5 Centrifuges

1. Before the rotor, to the BSC, all viral work should be finished and the BSC decontaminated. The only items left in the BSC should be the samples in their capped centrifugation tubes.
2. Spray the rotor with 70% ethanol.
3. Place the rotor in the BSC and add the sample tubes to the rotor.
4. Then replace the lid and seal the rotor before removing from the BSC.
5. Spray the rotor with 70% ethanol and wipe to dry completely.
6. Remove the rotor and place it in the centrifuge and spin as needed.
7. When the spin is complete, remove the rotor and spray it with 70% ethanol before placing it in the BSC.
8. Once in the BSC remove the lid and then remove the tubes.
9. Again spray the rotor inside and outside with 70% ethanol.
	1. If there is a spill or crack in one of the tubes. Wipe the inside of the rotor with 10% bleach. Be sure to mop up any access liquid and treat it as described in Section 8.
	2. Then wipe the inside with sterile distilled water to prevent rusting.
	3. Then spray the inside with 70% ethanol.
10. The rotor may now be removed from the BSC and stored.
11. The centrifuge must be decontaminated. Wipe the area with 10% bleach. Then wipe with sterile distilled water. Follow this by spraying the area with 70% ethanol to ensure complete decontamination.

6.2.6 Microscopes

Within Virus Room

1. Place lid on the plate containing the cells + virus.
2. Carefully wipe the outside of the plate with 70% ethanol.
3. Seal the lid to the plate with a strip of parafilm.
4. Remove the plate from the BSC and place it on the microscope.
5. Use the microscope as needed.
6. When work is finished, gently wipe the plate with 70% ethanol, and return to the BSC.
7. Wipe the microscope with 70% ethanol. Be sure to be gentle around the lens.

Outside Virus Room

1. Spray the inside and outside of a small sealed, leak-proof, lidded container with 70% ethanol before placing it in the BSC.
2. Place lid on the plate containing the cells + virus.
3. Carefully wipe the outside of the plate with 70% ethanol.
4. Wrap with parafilm.
5. Place the plate within the leak-proof container and seal the lid.
6. Spray the container with 70% ethanol.
7. The container may now be removed from the BSC and the room as described in Section 5.1. Use the microscope as needed.
8. When the work is finished, place the plate inside the leak-proof container and seal the lid.
9. Immediately spray down the microscope with 70% ethanol. Again, be sure to be gentle around the lens. If there is a spill, follow the procedures laid out in Section 7.
10. All supplies used to clean the microscope must be placed in a biohazard waste container.
11. Return leak-proof container to Virus Room. Spray outside with 70% ethanol.
12. Place the leak-proof container in the BSC and remove the plate. If work is finished with the plate, it may be returned directly to the incubator.

Note: When using a microtome, follow the same procedure. When the contaminated slices have been finished, it is important that all the slices be placed within the leak-proof container with any other contaminated items. The instrument may then be cleaned using 70% ethanol.

6.2.7 Biohazardous Waste

This section is site-specific. Please complete the procedure as appropriate for the waste procedures for your facility.

1. Tie off the top when the bag is ¾ full.
2. Place in a second biohazard bag.
3. Spray the outside of the bag with 70% ethanol.
4. Set aside and allow the outside of the bag dry.
5. Replace the biohazard bag in the bin.
6. Remove outer gloves and place them in the new biohazard bag.
7. Label the full bag as biological waste.
8. Process according to your site-specific procedures

**Note*:*** If the facility is responsible for its autoclaving, then clean and label as above. Place the waste bag in an autoclave safe tray with indicator tape on a cart. Proceed to autoclave the bags in a validated autoclave. After autoclaving, the bag should be tagged with a generator bar code and placed in the pickup area for disposal.

7. EMERGENCY PROCEDURES

7.1 Hazardous Spill Cleanup

**7.1.1 Minor Biological Spill:** A Minor Biological Spill is one that the laboratory staff is capable of handling safely without the assistance of emergency personnel. Typically, this is a volume of less than 5 ml. The decontaminant used is dependent on the biohazard spilled.

1. Alert people in the area of the spill and evacuate the area.
2. Remove and decontaminate any material splashed on you. Remove and decontaminate grossly contaminated clothing. Use a shower station if necessary.
3. Secure the affected area and post a biohazard warning sign. Wait 30 minutes for aerosols to settle.
4. Assess the situation, perform quick risk assessment, contact supervisor and/or colleagues for help if available (recommended to work in a team of two: one person can perform the spill cleanup and next person can be the clean person providing logistic support), don the appropriate PPE, and prepare fresh decontaminant for the cleanup operation.
5. Spill Cleanup Procedure
6. Define the area requiring clean-up and decontamination, allowing sufficient area for any splattering or drying which may have occurred.
7. Set up a disposal bag to allow easy discarding of contaminated cleanup materials
8. Set up a tray of fresh disinfectant and stack of rags or paper towels.
9. Saturate each rag/paper towel with disinfectant and then very gently place over the spill area
10. Start with the heart of the spill to prevent the spread or further aerosols
11. Move to the outer edge of the defined area and cover the area working from the outside of the center until the entire area is covered.
12. Let stand for appropriate contact time for the disinfectant reaction to complete
13. Gather absorbent to the center of the spill and place in a disposal bag.\*
14. Working from the outside in, use fresh absorbent to absorb any remaining liquid. Place used the material in the disposal bag.\*
15. Carefully remove glove and place in a disposal bag
16. Notify your PI, safety officer, and Safety & Risk Services at 604-822-2029 of the incident.
17. Report the incident on CAIRS (Centralized Accident Incident Reporting System).

\*Follow the disposal procedures for the building. Remember if bleach is used these materials cannot be autoclaved for 48 hrs. as they will cause an explosion

**7.1.1 Major Biological Spill**: A Major Biological Spill requires the assistance of safety and emergency personnel. While this is typically volumes > 5 mL, in some circumstances volumes less than this may be treated as a major spill if handling capacity is constrained by the nature of the hazard or injuries incurred.

1. Alert people in the area of the spill and evacuate the area.
2. Remove and decontaminate any material that has been splashed on you. Remove and decontaminate grossly contaminated clothing. Use a shower station if necessary.
3. Secure the affected area and post biohazard-spill warning signs.
4. Call for Hazardous Materials Response: 911.
5. Have a person knowledgeable of the incident and the laboratory assist emergency personnel.
6. Notify your safety officer, PI, and SRS of the incident.
7. Report the incident on CAIRS.

7.2 Loss of Electrical Power

If the Biosafety Cabinet (BSC) is connected to Emergency Power, it will continue to function or resume function in a power failure. If not on Emergency Power, work must be contained immediately.

1. Close all containers securely or decontaminate and disinfect materials that cannot be contained.
2. Leave the outer gloves in the BSC.
3. Close the cabinet sash.
4. Post a sign on the cabinet to keep the sash closed until power is restored. Then leave the room following proper exiting procedures, Section 4.
5. If there is a power failure during a centrifuge run, leave the centrifuge to stop. When it is safe to return, remove samples to the BSC.

7.3 Medical Emergencies

**Minor to moderate laceration injuries to hand and arms**

1. Stop work. Exit the room, doffing PPE.
2. In a handwash sink, place wounds under running water for 15 minutes and wash the affected area with disinfectant/soap.
3. If the bleeding persists, apply pressure to stop the bleeding and seek medical attention.
4. If the bleeding stops, bandage wound with a water-resistant bandage
5. Seek medical attention or call for first aid as necessary

**In the event of suspected or known exposure incident:**

Ideally, the laboratory staff, the PI, and the occupational health professionals should review the biological agent being used and develop a laboratory-specific exposure-response plan before an exposure incident..

1. Report the incident to your supervisor and the Biosafety Office (604 822 4353).
2. Record the details of the exposure incident including the route of exposure, the infectious agent and an estimate of the dosage.
3. Seek Medical Treatment.

**Major medical emergency, such as a cardiovascular event:**

* Dial 911 and then your supervisor.

7.4 Building Emergencies

**In the event of a fire in the lab**

a. Immediately suspend work in the BSC, seal open vessels, and close the sash.

b. Evacuate the building and proceed to your lab's designated meeting area outside of the building.

**In the event of a fire drill or other building evacuation notice**

1. Suspend work in the Biosafety Cabinet as soon as possible, lower the sash, and attach a sign to the cabinet to keep the sash closed.
2. Evacuate the building, as above.

8. SPILL PROCEDURE

8.1 BSC and Other Sensitive Equipment

1. Immediately leave the area and allow any aerosols to settle for 30min.
2. Carefully lay paper towels on top of the spill to collect the excess, and then add 0.1% SDS to the towels.
3. Generously spray the area down with 0.1% SDS.
4. Let spill + cleaner sit for 15 min untouched. Periodically adding more as needed to achieve contact time.
5. Push paper towels from the outer edge to centre of the spill. Place the paper towels in the biohazard bag within the BSC.
6. Generously spray the area down with 0.1% SDS again and allow it to sit for 15min.
7. Mop up the remaining liquid with paper towels and dispose in the biohazard bag within the BSC. Finish by wiping down with 70% ethanol.
8. When finished tie off the biohazard bag and place it in the biohazard waste.

8.2 Incubators

1. Immediately leave the area and allow any aerosols to settle for 30min.
2. Carefully lay paper towels on top of the spill to collect the excess, and then add 0.1% SDS to the towels.
3. Gently move any dishes to a rack that the spill did not contaminate.
4. Use paper towels to absorb any remaining liquid and prevent dripping from the rack during removal from the incubator.
5. Remove the rack from the incubator and transfer it to the BSC.
6. Wipe off the rack with paper towels soaked with 0.1% SDS.
7. Generously spray the rack down with 0.1% SDS and let sit for 15 min in the BSC.
8. After 15 minutes, wipe the excess liquid from the rack and the incubator.
9. Place the paper towels in the biohazard bag within the BSC.
10. Repeat steps b-h. Do a final wipe of the surfaces with 70% ethanol.
11. When finished tie off the biohazard bag and place it in the biohazard waste.

9. ANIMAL FACILITIES PROCEDURES

**Note: you must check with your animal facility to determine if lentiviral procedures are allowed.**

Lentivirus experimental procedures will have to be approved by the ACC, the Biosafety Committee and the animal facility manager. A general guideline for recombinant viral work in animal facilities is available at *BIO-SOP-009 Recombinant Viral Work in Animal Facility*. Every facility will have variations on this protocol in consideration of their facility design and operational practices. It is important to ensure not only the safety of yourself when working with Lentivirus but also the facility staff and other users.

It is also important to note that you will need to have Biosafety Certificate for the rooms that you are working in, as well as Active Animal Protocols.

10. MEDICAL SURVEILLANCE AND TRAINING

10.1 Medical Surveillance

All staff and students working with the lentivirus system or other agents requiring CL-2+ containment must participate in a job hazard analysis through the medical surveillance program administered by UBC’s [Occupational and Preventive Health Unit](http://www.hr.ubc.ca/wellbeing-benefits/workplace-health/occupational-preventive-health/) (OPH). Medical surveillance services provided by OPH involve workplace risk assessments, vaccination, and personal health history reviews, and occupational health screenings with the fundamental aim to protect the health and wellbeing of the individual. The purpose of these services is to reduce or eliminate the risk of exposure to potentially harmful pathogens and occupationally acquired infections.

10.2 Safety Courses

1. Biological Safety
2. Chemical Safety
3. Transportation of Dangerous Goods Class 6.2 (for individuals participating in shipping and receiving)
4. Any other applicable course for the research you are performing.

10.3 In House Training

Students and staff must be trained in handling, disposal, and emergency protocols. They must sign in writing that they have understood the training prior to starting the experimental work with the infectious particles. The training program should incorporate the following:

10.3.1 Lentivirus Risk Assessment

1. Modes of transmission to humans
2. Biohazard information
3. Safety precautions
4. Methods of disinfection and decontamination

10.3.2 Personal Protective Equipment

1. Equipment available, their location, proper use, and maintenance instructions
2. Operating principles of hoods and biosafety cabinets
3. Use and maintenance of pipetting aids
4. Use and maintenance of centrifuges

10.3.3 Universal Precaution Training

1. Assume everything in the lab is infectious
2. Handling and disposing of sharps
3. Pre- and post-work cleanup procedures

10.3.4 Emergency Response Training

1. Procedure for spills and leaks
2. Decontamination techniques
3. Fire response
4. Power failure response

10.3.5 Waste Disposal Procedure

1. Decontamination/disinfection methods
2. Analysis of decontamination/disinfection efficacy
3. Hazard communication requirements
4. Storage and disposal procedure

**Personnel must demonstrate proficiency in the practices and operations of the lab facility. Once proficiency is demonstrated, the lab assigned trainer will sign off on the training record and maintain the record in lab documents.**

Appendix A: Lentiviral Generation

***Know which generation of lentivirus you are working with to help to determine your risk factor.***

The term “Lentivirus” actually means a slow-acting virus characterized by a long interval between infection and the onset of symptoms. This means there are many retroviruses that fall under this category, including HIV-1 and SIV. The scientific community, however, has come to use the term “Lentivirus” for a viral vector adapted from HIV-1. This vector was developed as a means to integrate a gene of interest into the genome of a cell. HIV-1 was used as the model because it is able to infect both dividing non-dividing cells.

There have been various “generations” of the lentivirus, each increasing the safety of the virus. The first generation has few differences to HIV-1 (some of the viral genes have been removed and the envelope gene has been replaced with the VSV-G envelop gene). These constructs are only able to transfer the vector RNA or gene of interest. The absence of the viral gene inhibits the vector to one round of infection. There is a possibility during the recombination of the three different constructs that replication-competent retroviruses (RCRs) will be generated. RCRs present a hazard to the recipient since they are now able to replicate within the cells. This means that the gene of interest will be replicated in many cells. The use of the VSV envelope helps to reduce the risk of RCRs however, there is still a chance, and the VSV envelope allows the virus to infect most types of cells.

The second generation of lentivirus found that deletion of the accessory genes vif, vpr, vpu and nef from the packaging plasmid resulted in little change to the transduction efficiency but a significance in the possibilities of RCRs. The same safety features are present in the second generation, as with the first generation. Deleting the accessory genes inactivates the wild-type virus and the same is believed for RCRs. However, there is still a small risk of the virus recombining to form a replicating version of the virus, especially if an exposed individual is infected with another virus.

Third generation vectors contain a different env gene and lack the accessory genes vpr, nef, vif, vpu, and tat. The packaging plasmids were further altered using this system as well. A fourth plasmid was introduced to supply the rev gene. The elimination of the tat accessory gene greatly reduces the risk of RCRs thus making the third generation the safest to work with.

Most third generation systems and some second generations have self-inactivation features. A deletion in the U3 region of the downstream LTR in the transfer plasmid results in the inactivation of promoter activity which could lead to transcriptional activation of any genes downstream of the integration site.7 This works because the reverse transcriptase in the transduced cell transcribes the inactive U3 region into both the LTRs rendering them inactive as well.

Appendix B: RG- 2 Agents that Require CL- 3 Operating Procedures under Certain Conditions

| **Agent** | **Conditions** |
| --- | --- |
| Clostridium botulinum | High aerosol production or production of the toxin |
| Haemophilus influenza | High aerosol production or high production of the virus |
| Hantavirus | Clinical specimens for serology or PCR |
| Hepatitis B and C | High aerosol production or high concentration of the virus |
| Human Immunodeficiency Virus | High aerosol production or high concentration of the virus |
| Human-based Lentivirus | All manipulations |
| Lymphocytic Choriomeningitis Virus | High aerosol production, high concentration of the virus, or the manipulation of infected transplantable tumours, field isolates, and clinical materials |
| Neisseria gonorrhoeae | High aerosol production or high concentration of the bacteria |
| Neisseria meningitides | High aerosol production or high concentration of the bacteria |
| Parvovirus B19 | When work cannot be performed in a BSC |
| Simian Immunodeficiency Virus | High aerosol production or high concentration of the virus |
| West Nile Virus | Handling human and animal clinical samples |
| Influenza (H1N1) | High aerosol production or high concentration of the virus |

***Information sourced from the Public Health Agency of Canada PSDS sheets.***

Appendix C: Personal Protective Equipment

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Appendix D: Emergency Phone numbers

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| --- |
| **Common Numbers** |
| UBC Safety and Risk Services | 604 822 2029 |
| Poison Control | 604 682 5050 |
| Vancouver Fire Dept. ( Non-emergency) | 604 665 6010 |
| Fire, Police, Ambulance | 911 |
| R.C.M.P | 604 224 1322 |
| **UBC Point Grey Campus** |
| Hazardous Materials Response | 911 |
| First Aid | 822 4444 |
| Student Health Services | 822 7011 |
| UBC Hospital Urgent Care | 822 7222 |
| Campus Security | 822 2222 |
| **UBC Hospital and Health Sciences Centre (Koerner, Purdy and Detwiller)** |
| Hazardous Materials Response | 0000 |
| First Aid | 0000 |
| Student Health Services | 822 7011 |
| UBC Hospital Urgent Care | 822 7222 |
| Security | 822 7225 |
| Maintenance | 822 7523 |
| **VGH, Jack Bell, Willow Eye Care Centre** |
| Hazardous Materials Response | 84 |
| First Aid | 84 |
| Student Health Services | 822 7011 |
| Security | 03021 |
| **BC Centre for Disease Control** |
| First Aid | 604 753 0183 |
| Security | 03021 |

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