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1.0 PURPOSE

Lentiviral vector systems have become increasingly common in research because they can integrate stably into both dividing and non-dividing genomes and confer long-term transgene expression. Lentiviral vector systems are derived from HIV-1, and it is vital to consider their potential pathogenicity and develop appropriate biosafety strategies.

Lentiviral systems can potentially generate replication-competent retrovirus (RCR) and oncogenesis due to positional insertion. The newer generation of vectors have several features incorporated into them that enhance biosafety (for example, the removal of the tat gene needed for replication of wild-type HIV-1 or the separation of vector and packaging elements onto four or more plasmids). However, due to the ease with which these vectors can transduce human cells, it is pertinent to carry out a thorough risk assessment each time a new vector system is manipulated.

2.0 PROCEDURE

General Criteria for Risk Assessment of Lentivirus Vectors:

- The nature of the vector system and the potential for the generation of RCR using the vector components-
    - First generation vs third generation
    - Expression of viral accessory genes
    - Expression of tat regulatory gene
    - Intact 3' LTR
    - Strong promoters
- The nature of the insert(s) e.g. is the insert a known oncogene or toxic transgene.
- Vector titer and total amount of vector.
- Biological containment level of any animals that may be used.

Laboratory Containment Considerations:

- When using the newer generation of Lentiviral vectors, either BSL-2 or enhanced BSL-2 containment (the use of BSL-3 techniques, performed in a BSL-2 environment) is usually most appropriate. However, this should be determined only after completing a thorough risk assessment and in consultation with the Research Biosafety Officer. Please provide detailed protocol before starting virus work.
- Note that only BSL-1, BSL-2 and enhanced BSL-2 are permitted at the LKSKI. It is strongly advised that all Lentiviral work be done during regular working hours. This is to ensure the worker's safety in the event of an accident.
- All Lentiviral work will be performed in room 547. Access to this room, is by key card and access will only be given to those workers that have satisfactorily completed Lentiviral training. Please contact research biosafety officer for training.
- Housekeeping staff will be prohibited from entering the room to remove biohazard waste. Therefore, all users of the room must participate in the cleaning of the room.

Personal Protective Equipment:

- All persons working with Lentiviruses must wear a lab coat/gown with elasticized cuffs such that skin is not exposed. These lab coats are not to be worn outside of the virus lab and must be laundered on a regular basis. In the event of a spill onto the lab coat/gown, it must be autoclaved first, before being laundered.
- All workers must double glove when working with virus and the outer pair should be replaced regularly. The gloves must extend to cover the cuff of the lab coat. Do not spray gloves with 70% ethanol or other chemicals as this has been shown to increase the permeability of gloves, which compromises their protective ability.
- Laboratory appropriate clothing only is permitted in the Lentivirus room. No shorts, skirts, open toed shoes or sandals are permitted. Hair must be tied back and absolutely no eating or drinking is allowed.
- Eye protection and face masks must be worn.

- At the end of the experiment, lab coats/gowns must be removed, hands must be washed and the personnel must ensure that all doors are thoroughly closed and locked.

Prior to commencing experimental work:

- Book the biosafety cabinet on RFBMS.
- Turn on biosafety cabinet (BSC) and clean the inside surfaces with 70% Ethanol or diluted Virox.
- Allow the BSC to run for at least 5 minutes.
- Disinfect the exterior of all material going into the BSC with 70% Ethanol.
- Place biohazard bags, waste containers, paper towels, tape, a beaker and spray bottles containing freshly prepared 1:10 bleach/Virox in the BSC.
- All work in the hood must be conducted on absorbent pad (plastic side down). Ensure that enough pads are present in the BSC to cover the area of your work.
- **Note**- the use of sharps (glass pipettes, needles etc.) in the BSC is prohibited.

When using Lentiviral vectors

1. Avoid the generation of aerosols. The use of the BSC vacuum is prohibited as this is a likely source of aerosols.
2. Do not touch anything outside of the BSC with dirty gloves. Replace gloves with clean gloves, inside the hood, discarding the dirty gloves in the biohazard bag, before touching anything outside of the BSC e.g. an incubator or centrifuge.

3. All liquid waste must be thoroughly bleached inside the BSC before being discarded-
    i. Liquid waste is decanted into a large bottle containing at least 100 ml of undiluted bleach. Once all liquid waste has been generated and discarded, add an additional 100 ml, close off the bottle, ensure the exterior surface has been decontaminated with virox wipes, then place bottle on the right hand side of the sink. Ensure that the bottle has been labeled with your name, date and time at which the decontamination was started. Bottles should be left for 24 hours to ensure complete deactivation of the virus. Liquids that have been thoroughly bleached can be disposed of down the sink with large quantities of water. Please be considerate to others working in the room when discarding bleach as it is very volatile.
    ii. Pipette tips should be directly disposed into a beaker with 1:10 bleach and allowed to sit for at least 1h. The tips can be collected by draining the bleach solution through a sieve (provided in the virus lab). The tips are then transferred to the biohazard bag with the rest of the solid waste.

iii. All solid waste should be placed into a plastic bag in the hood. Serological pipettes should be placed back in their sleeves then into the bag. Once all solid waste has been collected, the bag should be sealed, placed into a second bag, which is then sealed and its exterior disinfected with 70% ethanol and virox wipes. This bag can then be thrown in the big grey biohazard waste collection bin outside the virus lab (ask BSO if you have a doubt)

4. After removal of all garbage from the BSC, it should be washed down with diluted Virox or virox wipes (provided). Allow the surface to remain wet for at least 30 seconds, and then wipe down.
5. Clean the BSC with 70% ethanol. Let the biosafety cabinet run for additional 5 min before turning it off.
6. Wipe down the exterior or the incubator, centrifuge, microscope, bench, taps with virox wipes.

Transport of Virus:

- Any cells that are with or are producing viral vectors should be carried to and from the BSC in a sealable container, lined with absorbent material. Inside of incubators, the lid can be opened to allow gaseous exchange.
- If cells have to be removed from room 547, it is advisable to only move them 3 days' post infection. This is the theoretical lifespan of the virus in solution. Before moving the cells however, remove all medium and thoroughly wash the cells (5 times), taking care to treat all washes as contaminated waste and decontaminate it according to the previous guidelines.

Use of Incubators:

- There are two incubators in the virus room. All users must book the incubators by using the log-sheet posted on the incubators. Add your name and contact details. Make sure the incubator has enough water.

- All work should be done in vented tissue culture flasks. However, if tissue culture plates are used, they must be stored in the incubators in sealable containers, which can be opened in the incubators to allow gaseous exchange.

- The incubators will be decontaminated and cleaned every 6 months.

Spill Procedure:

Small Scale spill

- Advise all those in the area that a spill is present.
- If the spill is on the lab coat remove it and leave in BSC.
- Remove gloves that might have touched the spill in the BSC.
- Allow 30 min for aerosols to settle.
- Ensure that you are wearing all necessary protective clothing, double gloves and eye protection.
- Gently cover the spill in paper towels soaked in bleach starting from the outside and working your way in.
- Add Virox or Virox wipes to the spill again working from the outside towards the center.
- Carefully discarding the paper towels and wipes into a biohazard bag. Dry any remaining liquid with more paper towels. Discard them as before.
- Apply more Virox to the area. Allow it to stand for 5 min then dry with paper towels, discarding as before.
- Wash area with 70% Ethanol. Dry with paper towels discarding them into waste as before.
- Discard outer gloves into waste bag then put fresh pair on.
- Autoclave biohazard waste bag immediately in the virus room.

Large Scale Spill (Code Brown):

In the event of a catastrophic failure of the ultracentrifuge or a spill of large volumes of Lentiviral Vector:

- Advice all those in the area that a major spill is present, then leave the area immediately.
- Leave your lab coat in the room and wash your hands thoroughly.
- Contact the Biological Safety Office (BSO) and advise them of the incident. Give your name, where you are and the location and type of spill.
- Wait for the BSO to arrive. Do not let anyone enter the room.
- Call 9-911- prompt administration of post-exposure prophylaxis (e.g., within one hour) will minimize the already low risk of HIV infection from Lentiviral vectors.

3.0 DEFINITIONS

Term/Acronym	Definition
RCR	Replication competent retrovirus
BSC	Biosafety cabinet
BSO	Biosafety officer

4.0 REFERENCES

https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/guidance/lentiviral-vectors/document.html

Version	Approval/Sub-approval body	Approval date
01	Research Biosafety Committee	January 1, 2015
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05		