Safety Guidelines for Work with Lentiviral Vectors

Introduction:

Lentiviruses belong to the Retroviridae family and are characterized by a long incubation period, hence their name "lenti", meaning'slow'. Lentivirusal vectors used in the lab are a result of vector modifications performed on FIV (feline immunodeficiency virus), SIV (Semian immunodeficiency virus), EIAV (equine infectious anemia virus) and others, mainly HIV (human immunodeficiency virus). Most original vectors are considered pathogenic to humans. The viral vector used in research is contained in a protein envelope derived from VSV-G (Vesicular stomatitis virus G) instead of the original HIV envelope. This protein envelope enables it to attack a broader range of cells, contrary to the HIV. In addition, the vector can penetrate the nucleus membrane and integrate into the DNA, contrary to retroviruses which cannot penetrate the nucleus membrane and must wait for cell division.

Thus, the lentivirus is an attractive tool in the research lab, used as an efficient method for introducing genetic materials into the cell and replacing damaged or missing genetic material. Lentiviruses enable efficient transfection, *in vivo* labeling, creation of transgenic animals, etc.

The use of Lentiviral vectors raises safety issues concerning employees and environment.

The main risks associated with work with Lentiviral vectors are:

- Generation of replication competent lentiviruses (RCL)
- Oncogenesis
- Vector mobilization

The virus can enter the body:

- Via skin –puncture or absorption through a cut, wound or irritated skin
- Via mucous membranes - eyes, nose, and mouth.

Lentivirus work must be performed in a BSL2+-competent lab, meaning that work must be performed in a BSL2 level with adoption of BSL3-level work procedures.

The main purpose of these instructions is to prevent employee exposure and/or environment infection by lentivirus droplets or aerosol, therefore **any work with lentivirus must be performed in the biological hood, in a BSL2+ lab.**

It is recommended using at least third-generation plasmids (a system which includes four plasmids) allowing the production of replication-deficient viruses, for example Invitrogen Virapower system (recommended by the NIH). This system incorporates several safety features as follows:

- Only three (3) genes are used in the vector system - gag, pol on one plasmid, and rev on another). The gene tat is not expressed in the system.
- HIV-1 Env is replaced by the VSV-G gene.
- The vector and other essential structural genes are separated onto 4 plasmids and added randomly.
- The vector is “self-inactivating” due to a deletion in the 3' LTR (U3)

Safety guidelines for working with Lentiviral vectors in the lab:

1. Any work with lentiviruses shall be performed in a BSL2+ lab
2. Mandatory equipment in the dedicated room:
    - A biological hood (Class II)
    - A sealed centrifuge and closed centrifuge tubes
    - A microscope
    - An incubator
    - Weights
    - Biosafety waste disposal equipment: bins, biohazard bags, etc.
    - Decontamination equipment: bleach, spray bottle, paper towels, etc.
    - A set of micropipettes.
3. Signs should be posted on the entrance door stating:
    - "Work with Lentivirus"
    - "Restricted entrance for authorized personnel only"
4. Before commencing work:
    - Wear a closed long-sleeved laboratorycoat (preferably disposable) and 2 pairs of gloves
    - Prepare decontamination solutions (see decontamination paragraph)
    - Prepare biohazard bag inside the hood and another biohazard bag in the biohazard waste bucket.
    - Use of sharps is not recommended; if you cannot avoid using sharps prepare a separate bucket for sharps' disposal.
5. Transfer all the disposable equipment, such as pipettes, bottles, plates, etc. to biohazard bags. Do not leave lentivirus waste in the room upon work completion.

6. Use filtered tips and pipettes. Collect the used tips in a plastic bottle (medium clean and dry bottle inside the hood). Dispose into the biohazard bag after you have closed the bottle.
7. Keep the culture room door shut while working with Lentivirus.
8. No other vectors or viruses are allowed in the biological hood during the work with Lentivirus.
9. Do not leave virus-containing solutions unattended in the biological hood or in the centrifuge.
10. Personal protective equipment, gloves and disposable labcoat, should be discarded in a biohazard waste bag.
11. If you used a fabric coat and not a disposable coat, discard it in a clean biohazard waste bag and place in an autoclave at a temp. of 121ºc for 30 minutes. This coat may be used only after the sterilization process. It is also possible to send it to laundry.
12. You must not leave the Lentivirus room to any other room wearing the clothes in which you worked with the virus. You may leave the room wearing a clean fabric labcoat and gloves.
13. Cells for virus infection should preferably be grown in flasks (not in plates).
14. Plates / flasks should be placed in the incubator on a secondary tray.
15. Pay special attention to avoid aerosols and splashes.
16. After completing the work in the biological hood, replace the gloves with new ones. Do not touch equipment or surfaces outside the hood with contaminated gloves used for working in the biological hood.
17. To visualize the cells under a microscope:
    - Close the bottle
    - Clean the bottle from the outside with paper soaked with 70% ethanol
    - Bring the bottle or the plate to the microscope on a dedicated secondary tray.
    - Upon work completion, clean the microscope using 70% ethanol
18. Biohazard bags must be closed (not sealed) and transferred to the biohazard bag placed in the dedicated bucket. Close the outer bag as well and transfer to:
    - A biological waste collection container – if the faculty waste is collectively decontaminated in the autoclave.
19. Decontaminte in an autoclave Small volume liquid waste (up to 500 ml) may be decontaminated in the biological hood using plastic bottles (500 ml medium bottle) to be filled 1/5 of the volume with the liquid waste, domestic bleach containing 3%-5% hypochlorite (final concentration of hypochlorite must be 0.5%), wait 30 min and pour the decontaminated liquid to the sink.
20. Large volumes of liquid waste may be decontaminated using a double trap. The collection bottle should contain domestic bleach with hypochlorite. The final concentration of hypochlorite in the full bottle must be 0.5%.

21. Upon completion, wash the rubber hose using a hypochlorite solution.
22. Wipe the biological hood, the incubator handle and the equipment you used with 70% ethanol.
23. After removing gloves, wash hands thoroughly with soap and water.
24. Centrifugation must be carried out in aerosol-sealed tubes in a centrifuge located in the lentivirus room according to the following instructions:
    - Fill the plastic tubes up to 75% of the tube's volume.
    - Meticulously clean outer wall of the tube using 70% ethanol.
    - Balance the tubes by weighing. If you need to transfer liquid from one tube to another, do it in the biological hood located in the lentivirus room. Do not forget to clean again the exterior of the tube before taking it out from the biological hood.
    - Following centrifugation, open the tubes inside the biological hood located in the lentivirus room.
    - Decontaminate the rotor using 70% ethanol, even in absence of a spill.
25. Should you need to use the ultracentrifuge located in another room, follow the following instructions:
    - Post a clear signage of the equipment door room: "Work with Lentivirus", containing the following details: work duration, your name and lab number, your mobile phone number.
    - Fill the plastic tubes up to 75% of the tube's volume.
    - Meticulously clean outer wall of the tube using 70% ethanol.
    - Balance the tubes by weighing. If you need to transfer liquid from one tube to another, do it in the biological hood located in the lentivirus room. Do not forget to clean again the exterior of the tube before taking it out from the biological hood.
    - Gently insert tubes into the metal bucket of the ultracentrifuge. Avoid splashes.
    - Cover the metal bucket using a suitable metal cap.
    - Meticulously clean the exterior of the metal bucket using 70% ethanol. Replace gloves and coat with fresh ones. Leave the infected personal protection equipment in the Lentivirus lab.
    - Take the sealed tubes to the centrifuge using a secondary receptacle.
    - Following centrifugation, open tubes only inside the biological hood located in the Lentivirus room.
    - Decontaminate the centrifuge buckets as well as the rotor using 70% ethanol even in absence of a spill.

Safety guidelines for working with Lentiviral vectors in the preclinical research authority facility:

Specific additions for working with lentiviral vectors in animals facilities

1. The person intended to perform trials on animals must hold a certificate showing completion of the course "Research principles in trials on animals".
2. Injections must be administered inside a level 2 biological hood or while using a 3M-8835 respirator and face shield.
3. Clipped needles should be preferred.
4. Animals should be contained during injection. (Consider anesthesia prior to injection (for example, when it is not possible to use clipped needles).
5. **Rearing conditions following injection**:
   - Following injection, meticulously sterilize the puncture area. Follow up healing of the puncture area for several days.
   - D**o not** keep mice in positive-pressure cages. Mice should preferably be kept in negative-pressure cages. It is possible to keep the animals in an isolated room retaining negative pressure and a BSL2 hood.
   - Following one week, if the injection area has healed, it is possible to release the animals from isolation (after changing the bedding).
6. **Handling cages and beddings:** prior to changing the growth substrate, place the cage into an autoclave, then discard the waste and rinse the cage. It is also possible to clean the cages inside a biological hood so that the bedding is placed directly into a biohazard bag to be sterilized in the autoclave. It is possible to clean the cages using diluted bleach: 0.5% hypochlorite . Rinse with water after 30 minutes. Cleaning with bleach will always be performed inside a biological hood. Required PPE when cleaning cages: a long-sleeved lab coat, protection gloves, safety glasses and a face shield.
7. Indicate on the cage that the animal was inoculated with lentivirus.
8. Comply with all the work rules concerning personal protective equipment, biological waste and sterilization described in the chapter "Instructions for work with Lentiviral vectors in the lab".
9. These instructions are also applicable to animals injected with cells (human or from other animals) previously infected with lentivirus.
10. The approval requires coordination with the head of the preclinical research authority.
11. Some of the above might change according to the lentiviral generation and the genes inserted.

Decontamination:

The main disinfectant used for a broad range of microorganisms - viruses, bacteria, yeast and mold - is hypochlorite, the active material is found in domestic bleach. The hypochlorite concentration in bleach is 3% - 5%. It is possible to purchase domestic bleach at the general warehouse.

1. Always keep an open bottle of unscented domestic bleach.
2. Keep a spray bottle of freshly prepared 0.5 % hypochlorite inside the biological hood (prepare fresh once a week).
3. Label the preparation date of the diluted solution.
4. Prepare a 70% ethanol sprayer.
5. Prepare a facial shield in the laboratory and at the animals' facilities.

Liquid waste decontamination

Decontamination of liquid waste, such as mediums and virus containing samples, will be performed using a final concentration of 0.5 % hypochlorite in . Incubate for one hour.

Solid waste decontamination

1. Dip used pipettes for 30 minutes in a dedicated container containing 0.5% hypochlorite. Siscarded to the biohazard bag. The solution volume in the container should be enough to cover the pipette tip.
2. Plates/flasks will be decontaminated using 0.5% hypochlorite for 30min.Discard to the biohazard bag.
3. Attention! In the long run, placing vessels containing bleach leftovers in the autoclave can cause corrosion inside the autoclave.

Spill decontamination:

*** Note – even a drop released from a pipette is considered a spill.

1. Wear a face shield to prevent splashes during decontamination.
2. Cover the splash using paper towels.
3. Carefully pour on the paper towels hypochlorite solution (3% - 5% hypochlorite in domestic bleach). Let it mix with the spill for about 30 minutes. In case it is not possible to use hypochlorite, for example because of concern for damage to the equipment: centrifuge, hood, etc., use 70% ethanol.
4. Collect the paper towels to a biohazard bag.

5. When broken glass is involved, do not collect paper towel and glass with bare hands. Use a shovel and a wiper.