DUKE UNIVERSITY VIRAL VECTOR CORE-
STANDARD OPERATING PROCEDURES FOR WORKING WITH VIRAL VECTORS
(REV. 1/2016)

Background

Lentiviral vectors are based on the Human Immunodeficiency Virus (HIV). Lentiviruses are a subclass of retroviruses which are able to infect both dividing and resting cells. Lentiviral vectors have been depleted of all the pathogenic viral genes to create a non-infectious version of the HIV. During transduction, there is a theoretic possibility that the lentivirus may convert to a replication competent state. Although this scenario is highly unlikely, monitoring for such a possibility is encouraged, since such a conversion could compromise laboratory safety.

Modes of Transmission

Lentivirus may be transmitted by:

• Penetration of the skin via puncture or absorption (thought scratches, cuts, abrasions, dermatitis or other lesions)

• Mucous membrane exposure of the eyes, nose, and mouth.

Containment Level

Work with Lentiviral vectors must be conducted utilizing Biological Safety Level 2 (BSL-2) practices and procedures as identified by Duke University –

Institutional Biological Safety Committee (IBC).

Approval

Duke Viral Vector Core approved by the Duke IBC with the protocol #11-0014-03 to generate Rabies virus (RV)-based system. Duke Viral Vector Core approved by the Duke IBC with the protocol #10-6147-01 to generate Lentiviral and Adeno-Associated vector-systems.

Facility Considerations

The Principal Investigator of the Viral Vector Core must designate a laboratory that fulfills the facility requirements as outline the CDC/NIH publications Biosafety in Microbiological and Biomedical Laboratories for Biological Safety Level 2 laboratories.

Engineering Controls

The following safety equipment MUST be used when working with Lentiviral vectors*:*

• Certified Class II Biological Safety Cabinets

• Sealed centrifuge rotors and/or safety cups

• Vacuum lines equipped with an in-line HEPA filter as well as a primary and secondary vacuum flash containing a 10% bleach solution.

Administrative Controls

Work with Lentiviral vectors carry out by trained personnel who directed by a competent scientist (the PI). Access to the laboratory is limited when the agent is in use. The laboratory is posted with Duke University Biohazard signage. The Standard Operating Procedures (SOP) for the planned procedures must be written and shall be present in the laboratory at all times. All staff involved with the handling and administration of adenoviral vectors **must** receive Biosafety training that covers safety procedures. It is the Principal Investigator's responsibility to identify the staff requiring this training, and to call the Biosafety office to schedule a training session.

Personal Protective Equipment

The following personal protective equipment MUST be worn when working with Lentiviral vector*s:*

• Gloves (consider double-gloving depending on the procedures being performed)

• Lab Coat

• Goggles

• Face shield

Special Handling Procedures

1. Cells exposed to *lentiviral* vectors may not be removed from the laboratory for experimental purposes unless inactivated by approved procedures.

2. If you need to aerate cultures, it must be done slowly and in a manner that minimizes the potential for aerosol creation. This action must be carried out in a class II biological safety cabinet.

3. When pouring and pipetting samples, it must be done gently and slowly and must be carried out in a class II biological safety cabinet.

4. Extra precautions must be taken when using sharps. Appropriate substitutes for sharp items must be used whenever they are available. No sharps (including needles and Pasteur pipettes) may be used for working with Lentivirus-infected cell cultures nor when harvesting virus pellets. Use plastic aspiration pipettes instead of glass Pasteur pipettes.

5. For Aspiration- Use a plastic vacuum flask with a second vacuum flask connected to it as a backup, with non-collapsible tubing capable of withstanding disinfection. To the second vacuum flask attach a hydrophobic and a HEPA filter (or combination filter) to ensure that nothing is sucked into the house vacuum system. These 3 items must be attached in series from the vacuum source in the hood or a vacuum pump

Decontamination/Clean-Up Procedures

All materials that have come into contact with *Lentiviral* vectors should be disinfected using a 1:10 bleach solution before disposal. Additionally, all work surfaces must be disinfected with a 1:10 solution of bleach once work is completed and at the end of the work day. (Note: A 15-minute contact time is required for decontamination)

Waste Disposal Procedures

1. Non-Sharp Waste- All cultures, stocks, and cell culture materials must be disinfected and autoclaved prior to being disposed of into a double red bag-lined biohazard box.
2. Sharps Waste-All needles, syringes, razors, scalpels, Pasteur pipettes and pipette tips must be disposed of in an approved, puncture resistant sharps container. Sharps containers must not be filled up more than 2/3 of their capacity.

Injury/Exposure Incident Procedures

1. Eye or Mucous Membrane Exposure from Splash or Aerosols- rinse a minimum of 15 minutes using eye wash and report the incident to your supervisor immediately.
2. Skin Contamination-Wash affected areas with soap and water for 15 minutes and report the incident to your supervisor immediately.
3. Needlestick and/or Sharps Exposure- Wash affected areas with soap and water for 15 minutes. **Immediately** notify your supervisor. He/she will complete the Post-Exposure Incident Report and submit it to the Department of Environmental Health and Safety within 24 hours of the exposure. Contact University Police at 8-2323 to arrange for appropriate medical attention.

Spill Response Procedures

The following steps must be taken when cleaning up a spill:

1. Stop, notify others and isolate the area!
2. Put on appropriate PPE (lab coat, gloves, eye and face protection).

3. Remove glass/lumps with forceps or scoop if applicable and place into a rigid, puncture resistant container.

4. Small spills-Place paper towels soaked in bleach directly on the spill and let soak for 20 minutes.

5. Wipe up area and discard towels in biohazard waste container.

6. Continue wiping area with paper towels soaked in bleach until the spill area is completely cleaned.

7. Discard all materials in biohazard waste container.

8. Wash hands thoroughly.

References

Biological Safety Principles and Practices, 3rd edition, 2000. ASM Press. Edited by Diane O. Fleming, Ph.D, and Debra Hunt, Dr.P.H.

Biosafety in Microbiology and Biomedical Laboratories, 4th edition, May 1999. Centers for Disease Control. www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4.toc.htm

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