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Retroviral Vectors

Background

The family of Retroviridae, consists of two subfamilies, and multiple genera within those subfamilies. Members of these genera are classified as simple or complex, depending on their integrative potential. Complex viruses include Lentiviruses, which are included in a separate document. This document focuses on simple retroviruses, such as alpha-, beta-, gamma-, delta-, and epsilonretroviruses, which will be combined and referred to retroviruses for the remainder of this document. Retroviruses are composed of a nucleocapsid containing two copies of single-stranded, positive-sense RNA. Retroviral systems are typically designed from murine retroviruses and can be grouped into one of three classes; ecotropic (infecting only murine cells), amphotropic (can infect human cells) or pseudotyped (vector particles express glycoproteins and can infect human cells). The most common pseudotyped virus utilizes a glycoprotein from vesicular stomatitis virus (VSV-g). Retroviruses have the ability to integrate DNA into the host genome of dividing cells, allowing for the viral DNA to be passed on to progeny cells after cell division. While viral genomic integration is essential to obtain a stable expression of the gene of interest, it may potentially contribute to insertional mutagenesis. The primary safety concern is the oncogenic potential due to integration of the gene of interest into host DNA as it has been observed in human gene therapy.

Some retroviral vectors can be pseudotyped with a murine envelope to allow infection of murine cells. This would decreased exposure risks as these viral vectors would have decreased chances of infected human cells. Other retroviral vectors can be pseudotyped using the different envelope proteins, such as Vesicular Stomatitis Virus (VSV) G protein. This increases the tissue tropism as the VSV-G protein binds to a broad range of cell types and species. This also increases risk of infection following an accidental exposure as the virus will be able to infect any cell at the site of inoculation. Pseudotyping retrovirus also increases the stability of the viral particles to enable concentration of virus via ultracentrifugation. The tropism and transgene must be considered when determining containment level.

Symptoms of Exposure

While replication defective Retroviruses do not cause disease in humans, they still have oncogenic potential.

Environmental Stability

Enveloped viruses are rapidly inactivated when exposed to drying environments. However, it remains prudent practice in BSL-2 to disinfect with a broad-spectrum disinfectant, such as sodium hypochlorite (i.e. 1:10 bleach solution). This solution must be made daily.

Modes of Transmission

Retroviruses are transmissible through injection (i.e. needle stick), ingestion, exposure to broken skin or contact with mucous membranes of the eyes, nose and mouth.

Host Range

Retroviral vector systems are usually based on murine viruses, but some have the potential to infect human cells as well. Retroviruses can infect dividing cells but not non-dividing cells.

Approvals

Experiments using retroviral vectors require IBC approval before initiation of experiments.

Test Methods for Replication Competent Virus

**If vectors are being obtained from a commercial supplier, please check the manufacturer's information as to the quality control concerning replication competent viruses. This information should be supplied with the IBC application.

Retrovirus vectors (ecotropic and amphotropic) can be tested by amplifying any replication-competent virus (RCV) in permissive cell lines and then screening by an appropriate replication competent retrovirus (RCR) detection assay (i.e. PG-4 S^+L^- assay, or the marker rescue assay). The vector stock should be tested at a sensitivity limit of 1 infectious unit per mL. (Wilson et al, 1997 and Forestell et.al 1996).

Murine Retrovirus (amphotropic or VSV-G Pseudotyped) vectors can be tested by marker rescue, antibiotic selection, $PG3S^+L^-$, PERT or infectivity RT-PCR assays. The viral vector stock should be tested at a sensitivity limit of 1 infectious unit per mL. Vectors used for *in vitro* studies must be tested every six months to ensure no replication competent particles are being produced.

Laboratory Practices

Generally, retroviruses are classified as a **Biosafety Level 2** (BSL-2) organisms. Retroviruses require BSL2 practices and procedures for all work with the virus and Animal Biosafety Level 2 (ABSL-2) for all animal manipulation as well as animal housing. At the discretion of the IBC, experiments may need to be conducted at Biosafety Level -3 (BSL3). In the IBC application, the PI must justify that the gene to be expressed is not particularly harmful, and include citations to support these statements.

1. No work with retroviruses is permitted on the open bench.

2. A certified Class II biosafety cabinet must be used for all manipulations including (but not limited to):
   - Pipetting
   - Harvesting infected cells for RNA
   - Purification of virus
   - Infection of cell culture
   - Infection of animals

3. Centrifugation must be done in closed containers with **sealed rotors or safety cups**. Safety cups are to be loaded and unloaded inside the biosafety cabinet.

4. All vacuum lines must be fitted with a HEPA filter (an example is the "Vacushield ™" inline hydrophobic filter, Product # 4402 from Gelman Science , Millex FH vacuum line protector Millipore (Fisher) cat # SLFH05010, or "HEPA-VENT™" inline hydrophobic filter, Catalog # 6723-5000 from Whatman).

5. All laboratory staff working with or supervising work with retroviruses must be made aware of the hazards associated with the work, required safety practices and procedures, and proper handling of the agent, as well as be current on required laboratory safety and biosafety trainings.

6. Animal carcasses must be placed in autoclave bags and be designated for infectious waste disposal.

7. Special training must be given to all animal husbandry personnel on retroviruses, the hazards associated with the work, required practices and procedures and proper handling of bedding, cage washing, and all other husbandry materials associated with the experiment. This training would be provided by animal facility supervisors in consultation with REHS.

8. Signs and labels (universal biohazard symbol) must be placed to indicate each area where retroviruses are used or stored (including biosafety cabinets, incubators, refrigerators, laboratory entrance doors, etc.) The signs should include the name of the agent, emergency contact information and a biohazard sticker.

9. All work and manipulations of retrovirus must be conducted in a certified Class II biological safety cabinet. If there are extenuating circumstances or a biosafety cabinet is unavailable, please contact REHS (at the numbers listed at the end of this SOP) as additional precautions may be required.

Personal Protective Equipment

1. Disposable gloves.

2. Disposable gown or equivalent when introducing vector into animals or performing necropsies. Lab coats are adequate for tissue culture manipulations.

3. Eye Protection.

Instructions in the Event of Employee Exposure

- **EXPOSURE FROM SPLASH OR AEROSOLS – INHALATION**

  Report the incident to your supervisor and refer to the Rutgers Emergency Action Plan for further instructions. The supervisor should submit an incident report through https://MyREHS.rutgers.edu to document the event.

- **EXPOSURE FROM SPLASH OR AEROSOLS – EYE CONTACT, SKIN AND/OR MUCOUS MEMBRANE**

  Rinse a minimum of 15 minutes in eye wash or flush area with water, report the incident to your supervisor and refer to the Rutgers Emergency Action Plan posted in the lab for further instructions. The supervisor should submit an incident report through https://MyREHS.rutgers.edu to document the event.

- **NEEDLESTICK AND/OR SHARPS EXPOSURE**

  Contaminated skin should be thoroughly scrubbed for several minutes with soap or a 10% povidone solution (Betadine) and copious amounts of water. Report the incident to your

supervisor and REHS immediately after scrub. Seek medical attention at Campus Employee Health Services/Occupational Medicine Services. Refer to Rutgers Emergency Action Plan posted in the lab for after-hours exposure. The supervisor should submit an incident report through https://MyREHS.rutgers.edu to document the event.

- **EMERGENT EXPOSURES**

  For situations in which exposure to AAV occurred and medical treatment is an emergency, personnel should report to the Emergency Room, and ensure their supervisor completes incident report through https://MyREHS.rutgers.edu to document the event.

Decontamination

The most effective disinfectant against retroviruses is a 1:10 sodium hypochlorite (bleach) solution that is made fresh daily.

- To make this solution, dilute 1 part bleach to 9 parts tap water.
- Ensure a 15 minute contact time.
- Use this disinfectant for treatment of reusable equipment, surfaces, and liquid waste (final volume 1% bleach).

Disinfectant alternatives include 70% ethanol.

- Autoclaving for 1 hour at 121°C or 250°F (15 lbs psi of steam pressure).
- Use this disinfection method for reusable equipment, liquid waste or solid waste.

Animal Practices

1. When animals are infected with retrovirus, an Animal Biosafety Level - 2 (ABSL-2) area must be used and approved by the animal facility staff and REHS for the procedure. Concurrent approvals are needed from the Institutional Biosafety Committee (IBC) and the Institutional Animal Care and Use Committee (IACUC).

2. All bedding, waste and animals infected with retrovirus shall be treated as biohazardous. Cage changing and husbandry must be performed according to the hazard sign provided by REHS. All waste must be decontaminated by autoclaving or chemical disinfectant prior to disposal.

3. Animal carcasses must be placed in autoclave bags and be designated for infectious waste disposal.

4. All necropsies must be performed in a designated room using animal BSL-2 practices and procedures.

5. The following information must be posted on the door of the animal room. REHS will provide a sign template to the animal facility staff for this purpose.
   - A description of special housing required to ensure safety of animal facility personnel, such as ventilated cabinets or filtered cages.
   - A label on the animal cage indicating the hazardous materials to be administered to live animals. (i.e., retrovirus vector)

- The name of individual(s) responsible for handling the materials (i.e., Drs. X, Y and Z and Technicians A and B as per protocol #00000) and emergency contact information
- A description of how to handle animals, carcasses, and contaminated cages and bedding

References

- Braun, A. 2006. "Biosafety in Handling Gene Transfer Vectors." Current Protocols in Human Genetics. 12.1-12.18.
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- Forestell, SP, Nando, JS, Bohnlein, E and Rigg, RJ. 1996. Improved detection of replication competent retrovirus. J Virol Methods. 60:171-178.
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- Young, L.S., Searle, P.F., Onion, D., and V. Mautner. 2006. "Viral gene therapy strategies: from basic science to clinical application." J. of Pathology. 208:299-318.

Standard Operating Procedures

Acknowledgement Page

I, _____, have read the SOP for working with _____ Viral Vector. The following people will be conducting experiments using these vectors. The staff members know where to find a copy of this SOP in the laboratory and they understand the hazards and safe work practices as detailed therein.

Name	**Job Title**	**Initials**

Principal Investigator (print): _____

Principal Investigator (Signature):_____