

This Standard Operating Procedure is issued jointly by the University of Michigan Office of Research and the Department of Environment, Health & Safety to provide guidance on the safe use and handling of retrovirus and retroviral vectors.

RETROVIRUS/RETROVIRAL VECTORS: STANDARD OPERATING PROCEDURE

Containment Level

Retroviral vectors may be contained at varying biosafety levels, dependent on the nature of the inserted genes and its replication competence. Generally, retrovirus is classified as a risk group 2 (RG2) organism.

Approvals

Experiments using recombinant retrovirus require approval from the University of Michigan, Institutional Biosafety Committee (IBC) before initiation of experiments.

Precautions

1. Most researchers working with rodent cells use a retrovirus vector packaging system based upon a Moloney murine leukemia virus (MoMuLV) construct which has an ecotropic envelope. Viruses or vectors packaged with an ecotropic envelope cannot absorb efficiently to human cells and as such are not a significant potential hazard to humans.
2. Certain retroviruses have a different envelope coat, an amphotrophic envelope which broadens their host cell species range to human cells and various other species including rodents. Because of this, working with retrovirus vectors packaged with amphotrophic envelopes is a potential human hazard. Individuals working with such vectors should be well educated in the use of these vectors.
3. In addition, some of the different packaging cell lines are not absolutely devoid of replicating retrovirus. This could be in part because of recombination events which occur with endogenous retrovirus sequence. The effect of this potential problem is the potential to be working with retrovirus vectors which continue to be replicated and passed from cell to cell because of contaminating, replicating retrovirus.
4. There is a risk of insertional mutagenesis. The nature of the transgene or other introduced genetic element may pose an additional risk.
5. The replication-defective virus may become complemented in vivo. Complementation may cause replication and spread of an otherwise replication-defective vector
6. Biohazard warning signs and labels must be used to indicate each area where retrovirus is used or stored (including Biosafety cabinets, incubators, refrigerators, laboratory entrance doors, etc.).

Laboratory Practices

1. Personnel must have prior experience with retrovirus or must be provided with suitable and sufficient information, instruction and training on working with the agent prior to initiating work.
2. The room should be balanced negative in relation to surrounding spaces, including corridors
3. No work with retrovirus is permitted on the open bench.
4. A certified biosafety cabinet must be used for all manipulations including (but not limited to):
 - a. pipetting
 - b. harvesting infected cells for RNA
 - c. loading and opening containers
5. Only one individual may use a biological safety cabinet at any one time.
6. Centrifugation must be done in closed containers (i.e. safety containment cups) and using sealed rotors.
7. All vacuum lines must be fitted with a HEPA filter (an example is the "Vacushield™" in-line hydrophobic filter, available through laboratory supply catalogs).

Personnel Protective Equipment

1. Gloves (nitrile, latex, etc)
2. Wrap around outer clothing when introducing vector into animals or performing necropsies. Lab coats are adequate for tissue culture manipulations.
3. Goggles (not to be confused with safety glasses)

Decontamination – * Note - This is not to be performed for personnel exposure

1. The most effective disinfectant (with a minimum 15 min. contact time) is a freshly prepared 10:1 dilution of sodium hypochlorite (bleach) solution (stock is 5% solution).
2. Autoclaving for 1 hour at 121°C or 250°F (15 lbs per square inch of steam pressure)

Animal Use

Concurrent approvals are needed from the Institutional Biosafety Committee (IBC) and the Institutional Animal Care & Use Committee (IACUC).

If animals are administered retrovirus intravenously, you must work under the assumption that animals may shed the recombinant retrovirus, and take appropriate precautions as described in this section.

1. Perform inoculations carefully to reduce the possibility of creating splashes or aerosols. Use of a biological safety cabinet is highly recommended for administration.
2. When animals are administered retrovirus/retroviral vectors intravenously, an Animal Biosafety Level-2 (ABSL2) area must be approved and used for the housing of these animals for the first 72 hours after administration.
3. Infected animals may excrete retrovirus during this time. Caution must be observed when dealing directly with infected animals to avoid being bitten, clawed or scratched. Precautions must be taken not to create aerosols when emptying animal waste material and when washing down cages, or cleaning the room with pressure hoses. After this time, the animals must be changed to a clean cage and can then be moved to a standard animal housing facility.

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4. Special training must be given to all animal husbandry personnel on retrovirus, 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.
5. All necropsy within the first 72 hours shall be performed using BSL2/ABSL2 precautionary practices and procedures.
6. Arrangements must be made with EHS for proper disposal of animal carcasses.

Employee Exposure

1. Eye Exposure from splash or aerosols - rinse a minimum of 15 minutes in eye wash or flush area with water and report the incident to your supervisor immediately after flushing. Follow up at the university's occupational medical provider, MWorks (998-8788). After hours refer employees to the UMHS Emergency Room.
2. Needle stick and/or Sharps Exposure – Contaminated skin should be thoroughly washed using soap and water for approximately 20 minutes. Report the incident to your supervisor immediately after washing. Seek medical attention at the university's occupational medical provider, MWorks (998-8788). After hours refer employees to UMHS Emergency Room.