

# Tel-Aviv University –Safety Unit

## Standard Operating Procedure for Working with **Retroviruses / Murine Leukemia Virus (MLV) Vectors** in Animals

<p>1. Health hazards</p>	<p>Retroviruses consist of a varied family of enveloped RNA viruses with positive-sense RNAs that replicate in a host cell through the process of reverse transcription.</p> <p>Retroviruses belong to the Retroviridae family. The family Retroviridae is classified into the two subfamilies: Orthoretroviridae and Spumaretroviridae. The Orthoretroviridae subfamily includes six genera (<math>\alpha, \beta, \gamma, \delta</math> and <math>\epsilon</math> retroviruses, and lentiviruses). The first five genera possess oncogenic potential (referred as oncoretroviruses).</p> <p>Retrovirus vector systems are typically based on murine viruses - most commonly, these systems include ecotropic viruses (which can infect only murine cells), amphotropic viruses (which can infect human cells) or pseudotyped viruses, when vector particles express glycoproteins (GPs) derived from other enveloped viruses (which can also infect human cells).</p> <p>The most common GP currently used is VSV-g, however there are newer pseudotypes being derived from viruses such as measles (Rubeola), Ebola and Marburg .</p> <p>Pseudotyping vectors often results in a higher Biosafety level. Containment for vectors with the ability to infect human cells (amphotropic) will usually be recommended at BSL-2/2+, whereas for ecotropic vectors with no ability to infect human cells, BSL-1 containment may be appropriate.</p> <p>The gammaRetroviruses consist of a large number of leukemia and sarcoma viruses, of mice, cats, primates, and other mammals. Also included in the genus is reticuloendotheliosis virus of birds, which causes immunodeficiency.</p> <p>Members of the Retroviridae family, which include <math>\gamma</math>-Retroviruses and Retroviruseses, are characterized by their ability to retrotranscribe their RNA genome into a cDNA copy, which is then stably integrated into the host cell genome.</p> <p>Using the retroviral systems allows for the stable, heritable integration of a specific nucleic acid sequence into the target cell's genome.</p>
<p>2. Designated Area</p>	<p>ABSL-2 facility.</p>
<p>3. Training</p>	<p>Practical experience with animal care/maintenance, as well as Retroviruses, is required.</p>
<p>4. Personal Protective Equipment (PPE)</p>	<p>Gloves (consider double-gloving), Eyes safety goggles, Lab coat, Disposable shoe covers and Animal handling gown.</p>

	<p>N-99 respirator mask covering the mouth and nose when not working in a Class II Biosafety Cabinet (BSC).</p> <p>Appropriate PPE should also be used for lower arms such as sleeve covers or securing gloves over the sleeves of laboratory coat.</p> <p><b><i>Personnel should not work with Retroviruses, if skin is cut or scratched.</i></b></p>
5.General Precautions for Animal Use	<p>Tools (as, syringe, blades and safety needles where possible) should be adapted for BSL-2. Have a sharps container in close vicinity.</p> <p>Animals should be restrained or anesthetized during injection.</p>
6. Environmental / Ventilation Controls	<p>Work should be conducted in ABSL-2 facility, over absorbent pads in a class II type A1 or A2 biological cabinet.</p> <p>Retroviruses may be transmitted by :</p> <ul style="list-style-type: none"> <li>• Penetration of the skin via puncture or absorption (through scratches, cuts, abrasions, dermatitis or other lesions)</li> <li>• Mucous membrane exposure of the eyes, nose, and mouth (through direct contact or aerosols).</li> </ul>
7. Animal handling practices	<ol style="list-style-type: none"> <li>1. Animals must be housed in filter top cages marked as biohazards (including the name of the pathogen/biohazard). Handling the cages (including bedding) will be done only by the researchers.</li> <li>2. Use a class II Biological Safety Cabinet at all times (especially during injection or any surgical procedure), when performing work on these animals and/or when moving animals from dirty to clean cages</li> <li>3. Infected animals may shed Retroviruses for 72 hours after treatment; take precautions to avoid the creation of aerosols when changing or washing cages, or cleaning the room</li> <li>4. Dead animals must be placed in primary plastic bags, which are then placed in biosafety bags for infectious waste incineration</li> <li>5. All surfaces and racks that may be contaminated will be decontaminated with 0.6% bleach or virusolve ASAP</li> <li>6. When changing cages, use a standard microisolator technique: <ul style="list-style-type: none"> <li>place the cage containing the animals, under the biological safety cabinet and transfer the animals into a clean cage.</li> <li>spray the dirty cage with virusolve, remove from the safety cabinet and place on a transfer rack.</li> <li>when all cages have been changed, spray the dirty cages and rack again with virusolve, and cover the rack. Put on a pair of new gloves and bring the rack directly to the autoclave in the dirty cage wash area.</li> <li>immediately autoclave the dirty cages (1 hour at 121°C/250°F, 15psi of steam pressure). Once the autoclave cycle is completed, the cages can be emptied and the bedding disposed of in a normal fashion.</li> <li>**In cases where the use of autoclave (within the animal facility) is not an option: the cages (bedding) must be emptied inside the BSL-2 cabinet, directly to a double biohazard bag.</li> <li>Before closing the bags, carefully, add a small amount of water (250ml) to improve the sterilization process.</li> </ul> </li> </ol> <p><b><i>Do not close the bag completely/tightly (in order to avoid entering of steam during the sterilization process).</i></b></p>

	<p>Spray the dirty bag with 0.5% bleach or virusolve.</p> <p>Remove from the safety cabinet and place on a transfer rack/container.</p> <p>Put on a pair of new gloves and bring the rack/container, directly to the collection point of your department.</p>
8. Decontamination	<p>Decontaminate work areas with 0.6% bleach (0.6% active ingredient-Sodium hypochlorite) or virusolve for 30 minutes. Follow with water.</p> <p>Retroviruses are susceptible to: 0.6% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde, iodine solutions containing ethanol.</p> <p>Freshly prepared 10% household bleach (0.5% Sodium hypochlorite) recommended</p>
9. Spill and Accident Procedures	<ol style="list-style-type: none"> <li>1. Evacuate area, remove contaminated PPE and allow agents to settle for a minimum of 30 minutes. Initiate spill response procedure.</li> <li>2. Cover the spill with absorbent material. Starting at the edges and work towards the center.</li> <li>3. Carefully pour disinfectant over the absorbed spill, again starting at the edges. Saturate the area with disinfectant.</li> <li>4. Allow sufficient contact period to inactivate the material in the spill. Non-viscous spills require 15-20 minutes; viscous spills require 30 minutes.</li> <li>5. Use paper towels to wipe up the spill, working from the edge to center. Use tongs or forceps to pick up broken plastics, glass or other sharps that could puncture gloves</li> <li>6. Discard absorbent material in Chemical waste bags.</li> <li>7. Clean the spill area with fresh paper towels soaked in disinfectant. Thoroughly wet the spill area, allow to disinfect for 15-20 minutes longer, and wipe with towels.</li> <li>8. Discard all cleanup materials (soaked with disinfectant) in Chemical bag, and any contaminated PPE (pay special attention to gloves and shoe covers) in a biohazard bag. Close and secure the bags.</li> <li>9. Place bag in a second biohazard bag, secure and disinfect by autoclaving.</li> </ol> <p><u>Exposure:</u></p> <ol style="list-style-type: none"> <li>1. In case of skin contact or injection with Retroviruses, wash the affected area with soap and water for at least 15 minutes. Consult with Employee Health Center.</li> <li>2. For eye exposure, flush with water for at least 15 minutes. Consult with Employee Health Center. Report incident to supervisor. Supervisor reports the accident/injury to the Biosafety Unit.</li> </ol>
10. Waste Disposal	Autoclave all waste (1 hour at 121°C/250 °F, 15psi of steam pressure).
<p>I hereby confirm that I have read the SOP (Standard Operating Procedure) for Working with Retroviruses / Murine Leukemia Virus (MLV) Vectors in Animals, and agree to follow these procedures.</p>	
Name:	Title:
Signature:	Date:

