Institutional Biosafety Committee - UTEP	
Title: **IBC Standard Operating Procedure – Retrovirus Vectors**	
Date in Effect: 8 October 2025	**Revision Date:**
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A. Background

Retroviruses are enveloped, single-stranded RNA viruses that replicate through a DNA intermediate using reverse transcriptase. Commonly used retroviral vectors (e.g., based on Moloney Murine Leukemia Virus, MMLV) are employed to stably integrate transgenes into the host genome, primarily in dividing cells. Because of their ability to induce permanent genetic modification, retroviral vectors are widely used in gene therapy and experimental research.

However, their integration into the genome carries a risk of insertional mutagenesis, which may activate oncogenes or disrupt tumor suppressors. For this reason, retroviral vectors—especially those expressing oncogenic, immunosuppressive, or toxic transgenes—must be handled using rigorous biosafety measures.

B. NIH Guidance

Under the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, retroviral vectors are classified as Risk Group 2 (RG2) biological agents. Their use requires containment at Biosafety Level 2 (BSL-2) for standard applications. Enhanced containment (BSL-2+) is recommended when working with VSV-G pseudotyped viruses, oncogenic transgenes, or replication-competent retroviruses (RCR). Risk assessments must be completed for all vector systems and included in the Institutional Biosafety Committee (IBC) protocol application.

C. Health Hazards and Risks of Retrovirus Vectors

The primary hazard associated with retroviral vectors lies in their potential to integrate into the host genome, which may cause insertional mutagenesis. Although replication-defective systems are commonly used, failure in vector production controls may lead to the formation of replication-competent retroviruses, posing additional risks. Furthermore, the use of human-derived cell lines in vector preparation can introduce human pathogens.

<u>Potential Health Effects:</u>
Accidental exposure to retroviral vectors can result in the integration of vector DNA into the genome of exposed individuals. This may cause oncogenic transformation of cells. While acute symptoms are rare, the long-term effects, such as cancer development, are a concern. In some cases, exposure may result in flu-like symptoms due to immune system activation.

Post-Exposure Protocols:
In the event of exposure, the affected area should be flushed immediately with water for at least 15 minutes. Eye exposures require rinsing at an eyewash station, and needlestick injuries must be scrubbed thoroughly with soap and water. All exposures must be reported promptly to the supervisor and Environmental Health and Safety (EH&S), and an official incident report must be completed. Medical evaluation should be sought to assess risk and determine if further monitoring is necessary.

Personnel exposed to retrovirus should report to their supervisor and the Emergency Department and Occupational Health and Safety. Contact the Biosafety Officer if you are unsure of the proper response and complete the EH&S Incident and Injury report form found at https://www.utep.edu/ehs/_files/docs/forms/ehs-injury-incident-report.pdf.

D. Biosafety Precautions

Containment Level:
Retroviral vector work should be conducted under BSL-2 containment. When vectors are pseudo typed with VSV-G, or contain transgenes encoding oncogenes or toxins, enhanced containment (BSL-2+) is required. This includes stricter access controls, more extensive PPE, and additional procedural safeguards.

Engineering Controls and PPE:
All procedures involving retroviral vectors must be performed within a certified Class II Biosafety Cabinet (BSC) to prevent aerosol transmission. Centrifugation must be done using sealed safety buckets or rotors to contain potential aerosols. Personnel must wear a lab coat or disposable gown, gloves (double gloves when warranted), eye protection such as goggles or a face shield, and a face mask or respirator if there is a risk of aerosol exposure. All PPE must be removed before exiting the laboratory and disposed of or cleaned according to BSL-2 waste handling procedures.

Decontamination and Waste Disposal:
Decontamination of surfaces and equipment must be performed using a freshly prepared 1:10 bleach solution or 70% ethanol with a minimum contact time of 30 minutes to ensure viral inactivation. All solid waste contaminated with retroviral material, including pipette tips, gloves, and culture flasks, must be placed in biohazard bags and autoclaved before disposal. Liquid waste should be chemically disinfected with bleach to a final concentration of 10% and held for 30 minutes before disposal into the sanitary sewer in accordance with EH&S policy. Regulated Medical Waste (RMW) bins must be used for all materials contaminated with human-derived vectors or animal waste.

E. Animal Work with Retroviruses

Containment Levels:

Animal work involving retroviral vectors should be conducted under Animal Biosafety Level 2 (ABSL-2) if the vector contains any oncogenic, toxic, or immunosuppressive transgenes, if immunocompromised or humanized animals are used, or if pseudo-typing with VSV-G is involved. Animal studies that involve non-replicating retroviruses, without hazardous transgenes, and no pseudo-typing may be approved under ABSL-1 conditions following IBC risk assessment.

Disposal of Soiled Bedding and Waste:
Soiled bedding, cages, and carcasses must be treated as biohazardous for a minimum of 72 hours following administration of retroviral vectors. Bedding must be collected in biohazard-labeled autoclave bags, sealed, and autoclaved prior to disposal. Cages should be disinfected before removal from the containment area. Carcasses must be placed in double bags and stored in a designated freezer until collected for regulated medical waste disposal.

F. Spill Procedures and Evacuation

Spill in ABSL-1 Area. If a spill occurs in an ABSL-1 setting involving a small amount of retroviral material without aerosol generation, evacuation of the area is not required. Cover the spill with absorbent towels, saturate with 1:10 bleach, and allow it to sit for 30 minutes before disposal. Personnel must wear gloves, a lab coat, and eye protection while cleaning. All waste materials must be disposed of in designated biohazard containers.

Spill in ABSL-2 Area. For minor spills in ABSL-2 settings, follow the same procedure as above using appropriate PPE and absorbent disinfectant. If the spill is large or may have generated aerosols (e.g., via centrifuge failure), immediately evacuate the area, post a warning sign, and wait 30 minutes before re-entry to allow aerosols to settle. Notify EH&S and the PI, then re-enter the area with enhanced PPE to perform disinfection. A spill report must be filed, and contaminated materials disposed of as biohazardous waste.

G. Laboratory Practices

All personnel must be trained on the risks and safe handling practices for retroviral vectors before beginning work. Retroviral vector use must be documented in approved IBC protocols. All vector stocks and working solutions must be labeled clearly with the biohazard symbol and relevant vector information. Emergency contact information and spill response materials should be available and posted in each lab. Access to emergency eyewash and safety shower stations must be confirmed before initiating any work with retroviruses.

H. References

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH, 2023) - https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf

Cornell University EHS – Lentiviral Vector BARS Sheets (2024)

CDC & OSHA Bloodborne Pathogen Standard (29 CFR 1910.1030)

Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th Edition (CDC, 2020)

UTEP EH&S Incident Report Form: https://www.utep.edu/ehs/_files/docs/forms/ehs-injury-incident-report.pdf