

Case Western Reserve University Institutional Biosafety Committee

Policy and procedures regarding the use of lentivirus vectors

In accordance with *National Institutes of Health Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)*, all research performed under the auspices of the University that utilizes lentivirus based vector systems must first be approved by the Case Western Reserve University's Institutional Biosafety Committee (IBC). The following policy is consistent with the recommendations of the NIH Recombinant DNA Advisory Committee (RAC).

Risk Assessment

The following is a list of considerations important to determining the proper containment for research involving lentivirus vectors:

- the nature of the vector system and the potential for regeneration of replication competent virus(es) from the vector components;
- the nature of the transgene insert;
- the vector titer and the total amount of vector;
- the inherent biological containment of the animal host, if relevant and
- negative Replication Competent Lentivirus (RCL) testing.

The Nature of the Vector System

The potential for generation of RCL from HIV-1 based lentivirus vectors depends upon several parameters, the most important of which are:

- the number of recombination events necessary to reassemble a replication competent virus genome, and
- the number of essential genes that have been deleted from the vector/packaging system.

Commercial 3rd Generation Lentiviral Vectors

The CWRU IBC strongly recommends investigators purchase commercial 3rd generation, 4-plasmid vector systems, which are likely to provide for a greater margin of personal and public safety than other vector systems.

Third generation systems utilize 3 helper plasmids: a packaging construct, a VSV-G construct and a Rev construct, along with a Tat-independent gene transfer vector, providing 4 separate plasmids in all. Additional biosafety features come from the elimination of the accessory gene Tat, a protein essential for replication of wild-type HIV-1. Vector systems that use more than 4 plasmids are becoming available with even higher levels of Biosafety but are still considered 3rd generation for our purposes.

Per RAC recommendations, the use of these 4-plasmid vector systems requires BSL-2 containment procedures without a requirement to perform RCL testing. Higher biological containment and/or RCL testing may be required if the inserted gene is a known oncogene, has oncogenic potential, is a toxin gene, or if high viral titers are expected.

Commercial 2nd Generation Lentiviral Vectors

Commercially available 2nd generation, 3-plasmid vector systems separate packaging and gene transfer functions into three distinct plasmids and lack certain viral accessory genes. These viruses frequently are made to express the vesicular stomatitis virus G (VSV-G) protein in place of viral Env to increase cell tropism.

2nd generation systems should be handled under BSL-2+ conditions. Biological containment requirements may be downgraded to BSL-2 after submitting to the IBC the results of a standard p24 ELISA kit showing no detectable RCL in the viral preparations. (Contact the IBC office for information regarding the assay, controls and submitting results.)

As with 3rd generation vectors, higher biological containment and/or additional RCL testing may be required if the inserted gene is a known oncogene, has oncogenic potential, is a toxin gene, or if high viral titers are expected.

Additional Vectors

All other vector systems, whether commercial or created in the laboratory, will be evaluated on a case-by-case basis.

Animal Experiments

Per RAC recommendations, the CWRU IBC requires all inoculations to be performed under ABSL-2 conditions, at a minimum. However, wild type and immune-compromised mice cannot support the replication of infectious HIV. Therefore, in many cases it is acceptable to house inoculated mice under ABSL-1 conditions 72 hours post infection and after a thorough cleaning, cage change and any biohazard labels removed.

This refers only to mice that cannot support HIV replication. Mice that have been engrafted with human cells or are permissive for HIV replication must remain under ABSL-2 conditions.

Non-murine animal systems involving lentiviral vectors will be considered individually.

Non-human Lentiviral Systems

Non-human lentiviral systems, such as Feline Immunodeficiency Virus (FIV), should be treated with the same precautions as HIV-derived systems. While these vectors do not inherently infect human cells, many of these systems contain heterologous envelope proteins to increase cell tropism and therefore may have the ability to transduce human cells. Non-permissive, mouse lines may be handled in the same manner as those inoculated with HIV based vectors.