

Research with Lentiviral Vectors at WCMC

Scope

This SOP is designed to establish a standardized system of information and biosafety level at WCMC when handling lentiviruses.

Current Guidelines

NIH Guidelines for Research involving recombinant DNA molecules, April 2002 Appendix B: RG3 for HIV-1 and HIV-2 viruses.

Section II-A-3 Comprehensive Risk Assessment:

- BSL2 containment for activities involving all blood-contaminated clinical specimens, body fluids and tissues from all humans, or from HIV- or HBV-infected or inoculated lab animals.
- Activities such as producing research-laboratory scale quantities of HIV or other BBPs, manipulating concentrated virus preparations, and conducting procedures that may produce droplets or aerosols, are performed in a BSL-2 facility, using the additional practices and containment equipment recommended for BSL3.
- Activities involving industrial-scale volumes or preparation of concentrated HIV are conducted in a BSL3 facility, using BSL3 practices and containment equipment.

Biosafety in Microbiological and Biomedical Laboratories, 4th edition:

Nonhuman primates or other animals infected with HIV or SIV be housed in ABSL2 facilities using ABSL2 special practices and containment equipment.

Use of 3rd Generation Lentiviral Vectors

Third generation lentiviral vectors are usually created in a transient transfection system in which a cell line is transfected with multiple plasmid expression systems. These include:

- (1) The transfer vector plasmid (portions of the HIV provirus)
- (2) The packaging plasmid or construct
- (3) A plasmid with the heterologous envelop gene (*env*) of a different virus.

The multiple plasmid components of the vector are put into a packaging cell which is then inserted into the HIV shell. These so-called split-configuration packaging cell lines require multiple recombination events to generate replication competent lentiviruses (RCLs).

These 3rd generation lentiviral vector systems are widely used to transfer genes in cell culture systems and live animals. A number of features are incorporated in the latest vector designs to enhance biosafety. These features include:

- Transgene: Non-oncogene; Vector and packaging components are distributed onto multiple plasmids that contain very little, if any, overlap or homology
- Deletion of viral genes (number of HIV genes is reduced to three (gag, pol and rev))
- Non-native viral *env* used in packaging system
- No expression of Tat (essential for lentiviral replication)
- Deletion in the 3' LTR that results in "self-inactivation"

Risk Factors Involved

The major risks involved with using HIV-1 based lentiviral vectors are:

- (i) generation of replication competent lentivirus
- (ii) the potential for oncogenesis through insertional mutagenesis.

These risks can be mitigated by the nature of the vector system or exacerbated by the nature of the transgene insert encoded by the vector.

Lab Research at WCMC requiring BSL2 Containment

Based on the above risk assessment, under certain conditions, the biosafety level for lentiviral research can be reduced from 'enhanced BSL2' to BSL2 containment. These conditions would include:

- Third generation lentiviral vectors with above mentioned features using non-oncogenic and non-toxic transgene for laboratory scale production of lentiviral vectors.

Lab Research requiring Enhanced BSL2 Containment

Research with lentiviral vectors which would continue to require an enhanced BSL2 containment would include:

- Large scale production of third generation lentiviral vectors
- Use of lentiviral vectors when the transgene is (i) an oncogene; (ii) a toxin producing gene; (iii) an antagonist of a tumor suppressor gene etc.

Animal Studies requiring ABSL1 Containment

Use of lentiviral vectors in a non-permissive host such as wild-type mice

Based on the risk assessment and in accordance with the RAC guidance document "Biosafety Considerations for research with lentiviral vectors" the biosafety level can be reduced from 'enhanced ABSL2' or 'ABSL2' to 'ABSL1' for lentiviral studies conducted in animals such as wild-type mice, which are not permissive for HIV-1. As a result, the potential for shedding of RCL from such animals is very low (even if RCL were present in the original vector inoculum).

To conduct studies on mice using lentiviral vectors the following procedure is recommended:

- The initial delivery of lentiviral vector should be performed under ABSL2 facility or under enhanced ABSL2 containment so as to reduce the risk of autoinoculation by the investigator
- Within 1-7 days the containment can be reduced from ABSL2 to ABSL1

Use of non-human lentiviral vectors such as FIV (feline immuno-deficiency virus) in a non-permissive host such as wild-type mice

- Mice are not permissive hosts for FIV replication. ABSL1 containment is acceptable for mouse housing and husbandry.

Animal Studies requiring Enhanced ABSL2 Containment

Animal studies which would continue to require an enhanced ABSL2 containment (because of the potential for replication of HIV-1) include:

- Animals engrafted with permissive cells (e.g., human cells) or mice lines that are permissive for HIV-1 replication (e.g., SCID mouse with human immune system)
- Animal hosts that are permissive for HIV-1 replication
- Non-human lentiviral vectors such as FIV (feline immuno-deficiency virus) containing heterologous envelope proteins e.g., VSV-G (which could extend the tropism of the vector so that these vectors can transduce human cells)

Testing of Lentiviral vectors

Safety testing of mice or embryos for generation of replication competent lentivirus (RCLs) is not required at this time.

References

1. RAC guidance document "Biosafety Considerations for research with lentiviral vectors: Animal studies"
2. CDC Biosafety in Microbiological and Biomedical Laboratories, 4th edition.
3. NIH Guidelines for Research involving recombinant DNA molecules, April 2002