FACT SHEET

Recombinant Lentiviral Vectors

The following provides information on the use and containment of recombinant lentiviral vectors. Investigators should use these guidelines as part of their risk assessment when planning experiments with these vectors and preparing applications to the Institutional Biosafety Committee (IBC). Note the listed containment levels are the minimum that should be employed with these vectors: some experiments, such as the expression of toxins or oncogenes, may require higher levels of containment. The appropriateness of the containment should be considered as part of the investigator's risk assessment and will be reviewed by the IBC.

NIH Risk Group

RG3
Lentiviruses are a subset of retroviruses, which are simple, enveloped single-stranded RNA viruses.

Biocontainement Level

The Recombinant DNA Advisory Committee of the NIH Office of Biotechnology Activities issued a report that reviewed biosafety issues relating to lentivirus vectors. This report advised that reduced biosafety level containment was appropriate in the laboratory setting for research involving the use of advanced lentivirus vector systems that 1) separated vector and packaging functions onto multiple plasmids, 2) were produced at laboratory scale quantities, and 3) lacked expression of oncogenic transgenes. They specifically recommended that 4-plasmid systems that met specific criteria could be used at BSL-2 without the need to assay for replication competent virus (RCV).

BSL-2 enhanced:
Oncogenic transgenes
Lentivirus vectors that incorporate transgenes with oncogenic potential must be generated and used at BSL-2 enhanced containment regardless of whether second or third generation systems are used.
Scale of production
Lentivirus vectors made at a level of production > 100 ml volume must be generated and used at BSL-2 enhanced containment regardless of whether second or third generation systems are used.
CRISPR/Cas9
Vectors that encode both the guide RNA and the Cas9 must be used at BSL2 enhanced containment. In addition, vectors designed to knock down expression of tumor suppressor genes (or that may knock down these genes because of off target effects) must be used at BSL2 enhanced.
Early generation systems
First, second generation or 3-plasmid lentivirus systems should be generated and used at BSL-2 enhanced. These systems generally have one packaging plasmid, which includes all the important packaging components: Gag, Pol, Rev, and Tat, an envelope plasmid and the transfer vector. In general, lentiviral transfer vectors with a wildtype 5' LTR need the 2nd generation packaging system because these vectors require TAT for activation.

The investigator may request a downgrade in biosafety level to BSL-2 following demonstration that virus preparations have no detectable RCV based on results of an accepted RCV assay as described below. A protocol modification requesting reduction in biosafety level and including data from the RCV test must be submitted to and approved by the IBC before any BSL-2 or ABSL-2 work can be performed.

BSL-2

Third generation or 4-plasmid system vectors may be generated and used at BSL-2, as may second generation lentivirus systems that use a self-inactivating vector (see below) The 4 plasmids of the third generation system include 2 packaging plasmids, an envelope plasmid, and a transfer plasmid. 3rd generation packaging system offers maximal biosafety but require the transfection of four different plasmids into the producer cells.

The main differences in the 3rd generation system are as follows:

- The Tat gene has been eliminated from the packaging completely
- Rev is expressed on a separate plasmid
- The 5'LTR of the transfer plasmid has been modified to include a conventional promoter and the U3 region of the 3'LTR has been deleted. This is termed a self-inactivating (SIN) vector and can be packaged by both 2nd and 3rd generation packaging systems.

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

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

Earlier vector systems (such as two-plasmid vector systems) may have a higher potential for generation of RCV. The IBC does not typically require testing for RCV when 4-plasmid (third generation) systems are used or when a SIN vector is used with a 2nd generation packaging system (see IBC guidelines for RCV testing).

Infectious to Humans/Animals

Yes

Route of Transmission

Lentiviruses are transmitted via direct exposure to infected bodily fluids, sexual contact, sharing unclean needles. Lentiviruses may persist lifelong due to their ability to integrate into the host chromosome and ability to evade host immunity. Lentiviruses replicate, mutate and undergo selection by host immune responses.

Laboratory Hazards

Risks include direct contact with skin and mucous membranes of the eye, nose and mouth, parenteral inoculation, ingestion.

Disease

The clinical manifestation of HIV infection includes non-specific symptoms such as lymphadenopathy, anorexia, chronic diarrhea, weight loss, fever, and fatigue. Can cause severe immunologic and neurological disease in hosts. The major risks associated with lentiviral vectors are insertional mutagenesis and local inflammation.

Treatment/Prophylaxis

NRT inhibitors, Protease inhibitors

Pathogenesis

Insertional mutagenesis. Can infect non-dividing cells including immune cells. Can infect non-target cells. Can persist lifelong. High mutation rates. Inappropriate expression of gene product. Rescue by other human pathogenic viruses

Replication Competent

Possible

RCV Testing

Can be performed by the investigator using a standard p24 ELISA kit providing the assay has a sensitivity of < 12.5 pg/ml. A positive control for virus infection is not required; the IBC does not want the investigator to work with infectious HIV-1

for this assay. However, the assay must contain a positive control for the ELISA itself in the form of p24 antigen.

Virus should be tested for RCV by serial passage of tissue culture supernatant on 293T cells for 3 passages with subsequent testing of supernatant from each passage for p24 antigen by ELISA.

Investigators who are not generating their own viruses from 2 or 3-plasmid system but are acquiring already constructed virus stocks from a commercial source that has documentation filed with the IBC of acceptable RCV testing will not be required to test for RCV.

Disinfection

Effective disinfectants require a minimum of 20 minutes contact time. Use one of the following:

- RECOMMENDED: Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach)
- 5% Phenol
- 70% Ethanol or Isopropanol

Animals

ABSL-2: Animals must be injected in a Biological Safety Cabinet. Animals will be maintained at ABSL-2 for the duration of the study. All bedding, waste and animals infected with EBV shall be treated as biohazardous. After all animals are removed from their primary enclosure immediately autoclave or treat with chemical disinfectant. After disinfection, dump the cage contents and begin cleaning the cage for re-use. All waste must be decontaminated by autoclaving or chemical disinfection prior to disposal. Animal carcasses must be placed in autoclave bags and be designated for infectious waste disposal. All necropsies must be performed in a designated room using animal BSL-2 practices and procedures.

Animal cages must be labeled with a biohazard sign.

On the fourth day following infection, animals injected with replication incompetent vectors can be transferred to ABSL-1 standard conditions. The animals will be transferred to a clean cage, and the ABSL-2 cage will stay in the ABSL-2 quarantine space for appropriate waste disposal and cleaning. Once animals have been transferred to ABSL-1, they can be handled as with other ABSL-1 animals. However, for rodents that contain any human cells or tissues, step down to BSL1 will generally not be allowed: determined by IBC.

ABSL-2 or ABSL-1 for xenografts of transduced human/animal cells. Determined by IBC.

Sources:
https://ehs.stanford.edu/reference/epstein-barr-virus-fact-sheet
https://www.dartmouth.edu/~ehs/biological/biosafety_docs/110_3_ibcviralvectorpolicy18.pdf

125 South Fort Douglas Blvd, Salt Lake City, UT 84113
801.581.6590 | oehs.utah.edu