University of Texas Health Science Center at San Antonio

STANDARD OPERATING PROCEDURES (SOP)

Lentiviral Vectors

Approved by the Institutional Biosafety Committee – Sept. 4, 2007

The University of Texas Health Science Center at San Antonio developed this SOP to establish a standardized system of information, practices and biosafety level assignment when handling lentiviral vectors.

1. BACKGROUND

The use of lentiviral vectors has been increasing because they allow highly efficient gene delivery in a wide variety of cell types. The major risks associated with lentiviral vectors are 1) potential generation of replication-competent lentivirus (RCL) and 2) potential oncogenesis. These risks can be mitigated or exacerbated by the nature of the vector system and the nature of the transgene insert.

2. PROCEDURES

2.1 Agents- Recombinant lentiviral vectors (based on HIV, SIV, or FIV).

2.2 Employees potentially at risk- Laboratory workers handling recombinant lentiviral vectors.

2.3 Laboratory hazards – Penetration through the skin via puncture or absorption (through scratches, cuts, abrasions, dermatitis, or other lesions) and mucous membrane exposure of the eye, nose, and mouth are considered as potential exposure pathways for lentiviral vectors.

2.4 Approval – Experiments using lentiviral vectors require the approval of the Institutional Biosafety Committee.

2.5 Biosafety Level Assignment – The following guidelines are provided to assist in the determination of the appropriate biosafety levels of experiments employing lentiviral vectors. Biosafety level assignment may vary depending on specific hazards and risks associated with the project, pathogenicity of the agent, vector systems employed, toxicity/oncogenicity of the gene to be studied, and suitability of laboratory facilities. Final biosafety level determination will be made by the Institutional Biosafety Committee (IBC).

2.5.1 General criteria for risk assessment of lentiviral vectors:

- The nature of the vector system and the potential for regeneration of RCL
- The nature of the transgene insert (e.g., genes with known oncogenic potential or human toxicity are considered as high risk.)
- The vector titer and total amount of the vector used
- The types of manipulations involved (e.g. ultracentrifugation or sonication can generate infectious aerosols.)

2.5.2 Features of lentiviral vectors considered to improve biosafety:

- The advanced vector systems that separated vector and packaging functions onto multiple plasmids are less likely to generate RCL and are considered as low risk.

- The feline immunodeficiency virus (FIV)-based vectors do not pose the risk of RCL to humans and are safer alternatives to HIV-based vectors.
- The lentiviral vectors with drug selection markers allow the selection of infected cells, eliminating the concentration of virus particles by ultracentrifugation, which could generate infectious aerosols.

2.5.3 Examples of biosafety level assignments:

- BL2 or enhance BL2 containment is often appropriate for the use of advanced lentiviral vector systems that segregate vector and packaging functions onto multiple plasmids. Enhanced BL2 containment may include attention to sharps (and use of safety needles where feasible) and the use of personal protective equipment intended to reduce the potential for mucosal exposure to the vector in addition to all the BL2 precautions.

- Activities such as manipulating concentrated virus preparations, conducting procedures with a high likelihood of droplet or aerosol formation (e.g. ultracentrifugation, sonication), or handling transgenes with oncogenic potential, are performed at enhanced BL2.

2.6 Work Practices

1. Depending on the nature of the experiment, access needs to be restricted when work is in progress. This may be accomplished through the use of a dedicated room for lentiviral work, signage, or mechanical means (i.e. cardreader) and may vary depending on the facility.

2. Principal Investigator is responsible for ensuring that all personnel demonstrate proficiency in the practices and operations of the facility prior to beginning work with the lentiviral vectors.

3. Biological Safety Cabinets are used for all manipulations involving infectious materials.

4. Centrifuge Safety Cups must be used for centrifugation outside of a biosafety cabinet. Safety cups must only be opened in a biosafety cabinet.

5. Common use equipment must be immediately disinfected after use.

6. Minors (individuals under the age of 18 years old) are not allowed to work with lentiviral vectors and are not allowed in the lentiviral tissue culture laboratory.

3. CONTAINMENT AND MANAGEMENT OF RODENTS (THAT DO NOT HOLD HUMAN TISSUES)

Lentiviral vectors cannot replicate (even in wild-type form) in rodents. Therefore, animal biosafety level 1 (ABSL-1) for long-term use in infected rodents is generally considered acceptable. However, at the time of transfer of lentiviral vector and/or lentivirally infected cells into the animals (e.g., by injection or surgery), the animals may still have infectious virus on their wound or body secretions that could be transmitted to research staff. In addition, animals reconstituted with human tissues are theoretically able to support the replication of RCL (see section 4). Unless otherwise specifically approved by the IBC, the use of lentiviral vectors for gene delivery in rodents, which do not hold human tissues, requires the following procedures:

1. Replication-incompetent lentiviral vectors with established 3 and 4-plasmid systems that pose minimal risk of replication-competent virus must be used.

2. The initial delivery of viral vector should be performed under laboratory BSL-2 conditions, in a procedure room, separated from where animals are housed. Animals should then be housed in filter top cages, in a designated ABSL-2containment area, for at least 72 hours. Exception: Upon recommendation of the IBC, animals injected/infected with preparations that are tested to be free of RCL may be housed at ABSL-1 containment. The ABSL-2 conditions will be arranged with the director or designee of LAR and the veterinary staff at each research facility. These will typically be provided as a temporary, quarantine-type ABSL-2 room or cubicle that the animals will be held in during the 72 hour period. In special cases in arrangement with veterinary staff, specified ABSL-2 containment animal racks may be used within an otherwise ABSL1 designated vivarium room.

3. There will be specific signage / labeling on each ABSL-2 cage stating 'ABSL-2 Biohazard Containment – Quarantine for Lentiviral Vector Research," and these cages will not be allowed out of the ABSL-2 containment space.

4. Once the period of potential infectivity is over, the containment level can be reduced to ABSL-1 provided this has been requested and approved by the IBC. The animals will be transferred to a clean cage, and the ABSL2 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 used within ABSL-1 facilities as with other ABSL-1 animals.

5. ABSL-2- cages are autoclaved or decontaminated before they are cleaned and washed.

4. CONTAINMENT AND MANAGEMENT OF RODENTS RECONSTITUTED WITH HUMAN TISSUES AND OTHER ANIMALS.

In general this category of animals, after infection with lentiviral vectors (or lentivirus-infected material) will be housed in ABSL-2 facilities using ABSL-2 special practices and containment equipment. Biosafety level may increase to ABSL-2+ or ABSL-3, depending on the risk of the project, including animal species, specific agent, experimental manipulations, and animal facility design. Under certain conditions, animals may be housed at ABSL-1.