

I. Introduction

This document provides essential information for Research Investigators regarding work with human pathogenic lentiviruses and lentivirus vector systems in the lab and in animal models. The IBC will determine the final Biosafety Level (BSL) and/or Animal Biosafety Level (ABSL) for work with lentivirus based on risk assessment and in accordance with the *CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*, the NIH DNA RAC Guidance Document *Biosafety Considerations for Research with Lentiviral Vectors*, and this *SDSU IBC Guidelines for Research with Lentivirus*. Specific requirements within these guidelines will be explicitly indicated in the BUA Approval Letter. Use the requirements specified in the BUA Approval Letter and indicated in the BUA Application as a training tool to inform all research personnel of the procedures for working with Lentivirus in the research laboratory or animal facility.

II. Lentivirus

Lentivirus is a retrovirus that includes the following human pathogens: Human Immunodeficiency Virus (HIV), Simian Immunodeficiency Virus (SIV), and Human T-lymphotropic Virus (HTLV). Laboratory acquired infection can occur through percutaneous or mucocutaneous exposure of human and non-human primate bodily fluids: blood, semen, saliva, tears, urine, cerebrospinal fluid, amniotic fluid, breast milk, cervical secretion, and tissue. While occupationally acquired HIV is generally from percutaneous exposure to blood, all human and non-human bodily fluids are presumed to be infectious and all specimens with blood, unfixed tissues or organs, bodily fluids and devices coming into direct contact with any of these materials should be handled as if infectious.

Work with blood-contaminated clinical specimens, body fluids, cell cultures and tissues must be performed in a **BSL 2** containment facility and work practices. Requirements for BSL 2 are indicated in the *BMBL: Section IV – Laboratory Biosafety Level Criteria*. Human serum from any sources that is used as a control or reagent in a test procedure should be handled at BSL 2 facility and work practices. Human T-lymphotropic virus 1 and 2 (HTLV-1 and HTLV-2) also requires a **BSL 2** facility and work practices.

Activities such as producing research-laboratory scale quantities of HIV or SIV, manipulating concentrated virus preparations, and conducting procedures that may produce droplets or aerosols, are performed with Biosafety Level 2 (BSL 2) facility using BSL 3 practices **PLUS** precautions specific to work with lentivirus (**BSL-2 + Lenti**) as described in Section IV of this document.

Work with wildtype HIV-1, HIV-2, HTLV-1, HTLV-2, and SIV involving large scale volumes or preparation of concentrated virus are conducted at **BSL 3** facility and work practices.

III. Lentiviral Vectors

The SDSU Institutional Biosafety Committee (IBC) will determine the BSL of work involving the use of Lentiviral vectors based on the *BMBL*, *NIH Guidelines*, and a risk assessment. The risk assessment will evaluate the potential for generation of replication/competent lentivirus (RCL) and the potential for oncogenesis from the transgene among other risk factors.

Work with lentivirus in the vector form will be at a minimum BSL 2 facility and BSL 3 work practices PLUS precautions specific to work with lentivirus (**BSL-2 + Lenti**) as described in Section IV of this document. Requirements for BSL 2 facilities and BSL 3 work practices are indicated in *BMBL: Section IV – Laboratory Biosafety Level Criteria* and *NIH Guidelines: Appendix G*.

IV. Laboratory Practices/Containment Equipment (BSL 2 + Lenti)

In addition to complying with the corresponding facility and work practice containment as specified in the *BMBL: Section IV – Laboratory Biosafety Level Criteria* and if applicable, *NIH Guidelines*, work practices and containment for lentivirus and lentiviral vector work designated at BSL 2 + Lenti requires the following:

- All vacuum lines must be fitted with a HEPA filter (e.g. “Vacushield” in-line hydrophobic filter, Product #4402 from Gelman Sciences)
- No work with Lentivirus is permitted on the open bench. A Biosafety Cabinet must be used for all manipulations including, but not limited to:
 - pipetting
 - harvesting infected cells for RNA/DNA/proteins
 - loading and opening containers
 - initial delivery of vector in animal hosts
- Centrifugation must be done in closed containers and using sealed rotors. Rotors must be opened in a biosafety cabinet
- Avoid needles and sharps where possible
- Reduce risk of exposure by reducing potential of replication competent lentivirus using one of the following methods:
 - Segregate the lentivirus into vectors and packaging replication functions onto four or more plasmids. The use of HIV – 3rd generation packaging system or HIV – 4th generation packaging system are recommended
 - Use non-native Env or a heterologous coat protein (e.g. VSV-G) in place of the native HIV-1 envelope protein (However, the use of the certain coat proteins, such as VSV-G may broaden the host cell and tissue tropism of lentivirus vectors, which will be considered in the overall risk and biosafety assessment by IBC)
 - Removal of genes essential for replication such as TAT
- Labels must be placed to indicate each area where lentivirus is used or stored, including, but not limited to, biosafety cabinets, incubators, refrigerators, laboratory entrance doors, etc.
- All procedures involving manipulation of the virus or viral vector or infectious materials must be conducted within a certified biosafety cabinet or other physical containment device
- BSL 2 + Lenti includes the use of the following personal protective equipment to reduce the potential for mucosal exposure, splash to the face, and exposure of hands:
 - Gloves
 - Wrap around outer clothing when introducing vector into animals or performing necropsies. Lab coats are adequate for tissue culture manipulations
 - Goggles (not to be confused with safety glasses)

- N-95 Respirator, to be used with concentrated titers and highly aerosolizing procedures outside of the Biological Safety Cabinet (contact EH&S for further information) or in ASBL 2 Facility
- Blood borne pathogen training is recommended for all employees working with lentivirus and lentiviral vectors

V. Animal Research with Lentivirus

When animals are infected with lentivirus/lentiviral vectors, an Animal BSL-2+Lentivirus (**ABSL-2+Lenti**) area must be approved and used for the procedure. Concurrent approvals are needed from the SDSU Institutional Biosafety Committee (IBC) and the SDSU Institutional Animal Care and Use Committee (IACUC). All necropsy must be performed in a necropsy room using ABSL-2 + Lenti precautionary practices and procedures. Requirements for ABSL-2 containment facility and work practices are indicated in *BMBL: Section V – Vertebrate Animal Biosafety Level Criteria for Vivarium Research Facilities*.

Some animals, such as wild-type mice, cannot support replication of infectious HIV-1. As a result, the potential for shedding of the virus from such animals is very low, but not obsolete. Precautions must be taken not to create aerosols when emptying animal waste material and when washing down cages, or cleaning the room with pressure hoses so as to minimize the risk of autoinoculation by the lab personnel/investigator. If there is no expectation of infection, the site of inoculation has been thoroughly cleansed, and the bedding changed, it may be acceptable to consider reducing containment from ABSL 2 to ABSL 1 within a few days (the specific time period can be specified by the SDSU IBC, and may vary anywhere from 1-7 days depending on local and experimental considerations). It is strongly recommended by the Institutional Biosafety Committee that the lab personnel be responsible for all animal husbandry practices during the **ABSL-2+ Lenti** following infection of the animal. After the allotted time, the animals can be housed in an ABSL-1 facility. Contact EH&S for Standard Operating Procedure for work with ABLS-2+Lenti.

Animals engrafted with human cells or animal hosts that are permissive for HIV-1 replication constitute a special case, in light of their potential to support replication of infectious HIV-1. Use of lentivirus vectors in these animals requires a continuous high level of containment at ABSL-2+ Lenti. **All animal husbandry practices will be conducted by the lab personnel/investigator following infection of the animal.** Animals should be housed and kept at ABSL-2+ Lenti until euthanized.

Special training must be given to all animal husbandry personnel on lentivirus, the hazards associated with the research, required practices and procedures and proper handling of bedding, cage washing, and all other husbandry materials associated with the experiment. Contact EH&S at (619) 594-2865 to schedule a group training.

VI. Precautions

The major risks to be considered for research with lentivirus vectors are:

- potential for generation of replication-competent lentivirus (RCL), and
- potential for oncogenesis.

These risks can be mitigated by the nature of the vector system (and its safety features) or exacerbated by the nature of the transgene insert encoded by the vector.

Non-human lentivirus vectors (e.g. feline immunodeficiency virus) require the same level of safety as human lentivirus vectors, since these vectors have the potential to transduce human cells, and thus have the potential to cause insertional mutagenesis.

The virus is a slow infecting disease with the onset of symptoms occurring years after initial infection. The virus targets immune cells, leading to immunodeficiency.

Transmission of the virus is through bodily fluids so precaution must be taken when working with animals containing the virus or virus vector.

VII. Employee Exposure

1. Mucous membrane exposure (eyes, nose, mouth) from splash or aerosols – rinse a minimum of 15 minutes in eye wash or flush area with water and take patient immediately to Sharp Rees-Stealy La Mesa. Notify prior to seeking medical attention the lab manager/principal investigator, biosafety officer or Human Resources.
2. Inhalation exposure from aerosols – take patient immediately to Sharp Rees-Stealy La Mesa. Notify prior to seeking medical attention the lab manager/principal investigator, biosafety officer or Human Resources.
3. Needlestick and/or Sharps Exposure – to prevent inoculation, take patient immediately to Sharp Rees-Stealy La Mesa. Notify prior to seeking medical attention the lab manager/principal investigator, biosafety officer or Human Resources.

Contact Information

Sharp Rees Stealy Medical Centers
Occupational Health Services
5525 Grossmont Center Drive
La Mesa, CA 91942
(619) 644-6600

VIII. Decontamination

The most effective germicides (with minimum 15 minute contact time) are:

- 1% Sodium hypochlorite
- 2% Glutaraldehyde
- 5% Phenol

Lentivirus is sensitive to heat, detergents, and formaldehyde.

IX. Integrated Pest Management

Integrated Pest Management (IPM) is an important part of managing a research facility. Many pests, such as flies and cockroaches, can mechanically transmit disease pathogens and compromise the research environment. Even the presence of innocuous insects can contribute to the perception of unsanitary conditions. For a detailed description of the University's IPM program, please refer to the *SDSU Biosafety Manual*.

X. Employee Right-to-Know

It is important that all lab personnel (even those not directly working with the virus) be informed and aware that Lentivirus is being used in the lab.

XI. Example of IBC Lentiviral Risk Assessment

Example 1: *In vitro* study A

Use of a 4-plasmid derived lentivirus vector encoding siRNA against Lck in primary human T cells.

Considerations

1. What is the amount of vector to be produced? Low <100ml
2. What is the nature of the vector? 4-Plasmid System
3. What is the nature of the insert? Non-oncogenic insert

Tentative Risk Assessment = BSL 2

Example 2: *In vitro* study B

Use of a 2-plasmid derived lentivirus vector encoding luciferase in a human cell line (a549 cells).

Considerations

1. What is the amount of the vector to be produced? Low < 100ml
2. What is the nature of the vector? 2-plasmid System (non-commercial)
3. What is the nature of the insert? Non-oncogenic

Tentative Risk Assessment = BSL 2 + Lenti

Example 3: *In vivo* study B

Use of a 4-plasmid derived lentivirus vector encoding brain-derived neurotrophic factor (BDNF) in mouse brain.

Considerations

1. What is the amount of the vector to be produced? Low < 100ml
 2. What is the nature of the vector? 4-plasmid System
 3. What is the nature of the insert? Non-oncogenic*
 4. What is the nature of the animal host? Non-permissive for HIV-1
- Tentative Risk Assessment = ABSL 2 + 7days, then downgrade to ABSL 1.

* Even though BDNF is a growth factor for neurons, it has no known oncogenic activity for skin or blood cells that might be the target of a potential needle stick. Hence, this insert would not automatically trigger a requirement for increase biocontainment.

