

## STANDARD OPERATING PROCEDURES (SOP) Lentivirus and Lentivirus Vector Usage

### 1. SCOPE

This SOP describes the biosafety considerations and work practices when working with human pathogenic lentiviruses and lentivirus vector systems at the University of Pittsburgh. Section 3 of this SOP provides guidance for working with human pathogenic lentiviruses while Section 4 gives instructions on using lentivirus vectors. This SOP incorporates recommendations from the *CDC Biosafety in Microbiological and Biomedical Laboratories*, 5<sup>th</sup> edition, the *NIH Guidelines for Research Involving Recombinant DNA Molecules*, April 2002, the NIH DNA Recombinant Advisory Committee guidance of October 2006, and the Institutional Biosafety (IBC) Committee at the University of Pittsburgh.

### 2. DEFINITIONS

**Lentivirus Agents**- Lentiviruses are a subset of retroviruses that have the ability to integrate into host chromosomes and to infect non-dividing cells. Lentiviruses include viruses such as human immunodeficiency virus (HIV), simian immunodeficiency virus (SIV), and human T-lymphotropic virus (HTLV) that can infect humans. SIV/HIV hybrid viruses also fall into this category. Other commonly used lentiviruses that are infectious to animals, but not humans, include feline immunodeficiency virus (FIV) and equine infectious anemia virus (EIAV).

**Lentivirus Vectors** - Lentiviral vectors consist of recombinant transgene sequences and viral packaging and regulatory sequences flanked by long terminal repeats. Lentiviral vectors are designed to be non-pathogenic and introduce specific genetic material into the DNA of a cell after infection. In general, plasmid vectors expressing the different lentiviral components are co-transfected into cells to generate a virus which has the capacity to undergo a single round of replication when infected into the target cell. .

**Employees potentially at risk**- Laboratory workers handling pathogenic lentiviruses, recombinant lentiviral vectors, naturally or experimentally infected laboratory animals, or clinical specimens potentially infected with lentiviruses.

**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 agents. Infection occurring via the respiratory tract has not been documented, and is unknown.

**Registration requirements** - All Principal Investigators (PI's) working with blood-contaminated clinical specimens, body fluids and tissues from humans, lentivirus inoculated or infected laboratory animals, or lentiviral agents must be registered with Environmental Health & Safety (EH&S). A registration document may be obtained from [www.ehs.pitt.edu](http://www.ehs.pitt.edu) or by calling EH&S at 624-9505. PI's working with recombinant lentiviral vectors or genetic material from lentiviruses must also be registered with the Institutional Biosafety (rDNA) Committee. A registration document may be obtained from <http://www.rcco.pitt.edu/rdna/> or by calling 412-383-1768.

### 3. PROCEDURES FOR WORKING WITH LENTIVIRUSES

**3.1 Biosafety Level Assignment Work Practice Guidance**— The following guidelines are provided to assist in the determination of the appropriate biosafety level and work practices to be used with lentivirus work. For work with lentiviruses, biosafety level assignment is based on the risks associated with the project, pathogenicity of the lentivirus and suitability of laboratory facilities. Final biosafety level determination will be made by the Institutional Biosafety (rDNA) Committee in consultation with the Biosafety Officer/EHS. The University Biohazards Committee will be consulted as necessary and must be consulted for all work being considered at the BSL-3 level.

3.1.1 Biosafety Level 2 (BSL-2) – Diagnostic specimens that contain human blood, body fluids or tissues can be handled and manipulated at the BSL-2 level. BSL-2 is also appropriate for generating and using lentiviral vectors, and handling animals and animal tissues, blood, body fluids and cell lines that are infected with lentivirus vectors, as long as they contain no oncogenes and meet other criteria as listed in Table 1. Work with lentiviruses such as FIV and EIAV that are not infectious to humans can be done at BSL-2.

3.1.2 Biosafety Level 2+ (BSL-2+) – Culture and production of human pathogenic lentiviruses (such as HIV), manipulating lentivirus-infected samples for research purposes, using lentiviral vectors that contain oncogenes or that are not replication incompetent (see Table 1), manipulating concentrated virus preparations, or conducting procedures with a high likelihood of droplet or aerosol formation, are performed in a BSL-2 facility, using the additional practices and appropriate containment equipment for BSL-3. This shall be known as BSL-2+ (see 3.2 for definition of critical points). In addition, work with SIV and SIV/HIV hybrid viruses must be done at the BSL-2+ containment level since these viruses are infectious to humans.

3.1.3 Biosafety Level 3 (BSL-3) - Activities involving industrial-scale volumes or industrial-scale preparation of concentrated lentiviruses are conducted in a BSL-3 facility, using BSL-3 practices and containment equipment. Industrial volumes generally involve greater than ten liters of culture, however, that distinction is to be used only as a guide and may vary depending on the work being done, the specific agent and associated risks. Any BSL-3 work must be approved by the University Biohazards Committee and EH&S.

3.1.4 Animal Housing. Nonhuman primates or other animals infected with lentiviruses are 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 specific hazards and risks of the project, including animal species, specific agent, experimental manipulations and animal facility design.

**3.2 Inspection requirements**— Following completion of appropriate registrations, laboratories are inspected by EHS to verify appropriate containment and practices. The criteria for BSL-2+ includes meeting all facility containment requirements for BSL-2, while following all BSL-3 practice requirements outlined in *CDC Biosafety in Microbiological and Biomedical Laboratories*, 5<sup>th</sup> edition, the *NIH Guidelines for Research Involving Recombinant DNA Molecules*, latest edition. The specific additional practice requirements for BSL-2+, above BSL-2, include the following:

- 3.2.1 An operations manual must be prepared for the BSL-2+ agent, and must include:
- Approval page signed by the PI and the Biosafety Officer, and also the animal facility director if ABSL-2+
  - Emergency contact numbers and procedures,
  - Agents used, and locations of use and storage,
  - Facility entrance requirements,
  - Training requirements and records,
  - Medical surveillance requirements, and proof that personnel have met them,

- SOPs for routine procedures, including handling, Personal Protective Equipment (PPE), decontamination and disposal of waste, etc.,
- Autoclave verification program, if autoclave is used for decontamination,
- MSDS for infectious materials and hazardous chemicals.

3.2.2 Access is restricted when work is in progress.

3.2.3 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 organisms.

3.2.4 Any vacuum line is protected with liquid disinfectant traps and/or HEPA filters.

3.2.5 Class II or III Biological Safety Cabinets (BSCs) are used for all manipulations involving infectious materials.

3.2.6 Centrifuge safety cups must be used for centrifugation outside of a biosafety cabinet. Safety cups must only be opened in a Biosafety Cabinet.

3.2.7 Continuous flow centrifuges, or other devices that may produce aerosols, must be contained within devices that exhaust air either through HEPA filters or directly outdoors and away from occupied areas and air intakes.

3.2.8 ABSL-2+ - Cages are autoclaved or decontaminated before they are cleaned and washed.

3.2.9 ABSL-2+ - Materials not related to the experiment (e.g., plants, animals) are not permitted in the animal room.

#### 4. PROCEDURES FOR WORKING WITH LENTIVIRUS VECTORS

**4.1 Biosafety Level Assignment Work Practice Guidance** – The following guidelines are provided to assist in the determination of the appropriate biosafety level and work practices to be used when working with lentivirus vectors that are pathogenic to humans. Biosafety considerations are based on vector system used, the potential for oncogenic activity, and scale of production. Guidance and restrictions for lentiviral vector work is described below and summarized in Table 1. Final biosafety level determination will be made by the Institutional Biosafety (rDNA) Committee in consultation with the Biosafety Officer/EHS. The University Biohazards Committee will be consulted as necessary and must be consulted for all work being considered at the BSL-3 level.

**4.2 4-Plasmid Systems.** It is strongly recommended that investigators use 4-plasmid (3<sup>rd</sup> generation) lentivirus vectors from commercial vendors. 4-plasmid systems provided by other investigators from within the University of Pittsburgh or from outside can be used if absolutely necessary. The IBC does not require testing for replication competent viruses (RCV) when 4-plasmid (3<sup>rd</sup> generation) systems are used that contain no oncogenes and laboratory production is less than 100 ml. These 4-plasmid system vectors may be generated and used at BSL-2 and at ABSL-2.

NOTE: 4-plasmid systems that incorporate transgenes with oncogenic potential, or are made at a level of production >100 ml must be generated and used at BSL-2+ containment regardless of whether second or third generation systems are used.

**Table 1. Summary of biosafety level requirements for lentivirus vector production and use**

Oncogenic transgene or >100 ml production	No. of plasmids	RCV testing	Vector production	Use of viral vectors in vitro	Use of viral vectors in animals	Use of virus-transfected cells in animals
Yes	Any number	None required	BSL-2+	BSL-2+	ABSL-2+	ABSL-2+
No	4 or more	Not required	BSL-2	BSL-2	ABSL-2	ABSL-2
	3	Elect to test for RCV	BSL-2+	BSL-2 if approved by IBC	ABSL-2 if approved by IBC	ABSL-2 if approved by IBC
		No RCV test	BSL-2+	BSL-2+	ABSL-2+	ABSL-2+

**4.3 3-plasmid (second generation) systems.** 3-plasmid lentivirus systems should be generated and used at BSL-2+. The investigator may request to conduct such research at BSL-2 following demonstration that virus preparations have no detectable RCV. An IBC protocol modification requesting BSL-2 conduct must be submitted and include the name of the RCV test used, and all data from the RCV test (see section 4.5 below). IBC approval of the modification must be received in writing before any BSL-2 work can be performed with these materials.

Approval for BSL-2 use with 3-plasmid systems will be specific to each virus preparation made. If separate lentivirus preparations containing different transgenes are generated, each must be tested and shown to be free of RCV as approved by the IBC.

**4.4 Replication Competent Virus testing.** Testing for RCV can be performed by the investigator using a standard p24 ELISA kit providing the assay has a sensitivity of  $\leq 12.5$  pg/ml. It is recommended that the [Magee-Women’s Research Institute Transgenic and Molecular Core](#) (412 641-2415) performs the RCV assay for individual investigators. This is done on a fee-for-service basis. 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. Optical density readings from each passage along with positive controls and/or standards should be submitted to the rDNA office.

**4.5 Exceptions to the requirement for RCV testing of 3-plasmid lentivirus stock.** Investigators who are acquiring 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. Such information must be included in the IBC Protocol application.

Investigators who are not generating their own viruses from a 3-plasmid system but are acquiring already constructed viruses from a University of Pittsburgh or other investigator should contact the IBC regarding requirements for RCV testing, which will be reviewed on a case-by-case basis.

## 5. SERUM SURVEILLANCE

Serum testing will be available to individuals with potential exposure to lentiviruses, per the University Serum Surveillance Program. See the Serum Surveillance Program SOP on the EH&S Website for additional information on this program.

## 6. APPROVAL

The University of Pittsburgh's Biohazards Committee, Institutional Biosafety Committee and EH&S have reviewed and approved this SOP as attested by the signatures of the Committee Chairpersons and the University Biosafety Officer.

Lee Harrison, MD

Biohazards Committee Chairperson

March 11, 2008

Date

Simon Barratt-Boyles, Ph.D.

Institutional Biosafety (rDNA) Committee Chairperson

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Date

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