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RESEARCH WITH LENTIVIRUSES AND LENTIVIRAL VECTOR SYSTEMS

1. SCOPE

This guideline describes biosafety considerations and work practices for work with human pathogenic lentiviruses and lentiviral vector systems at the University of Pittsburgh.

2. DEFINITIONS

2.1 Lentivirus(es): Lentiviruses are a subset of retroviruses that have the ability to integrate into host chromosomes and to infect non-dividing cells, and include human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) which can infect humans. Other commonly used lentiviruses that are infectious to animals, but not humans, include feline immunodeficiency virus (FIV) and equine infectious anemia virus (EIAV).

2.2 Lentiviral Vectors: Lentiviral vectors consist of recombinant or synthetic nucleic acid sequences and HIV or other lentivirus-based viral packaging and regulatory sequences flanked by either wild-type or chimeric long terminal repeat (LTR) regions.

2.2.1 Replication-Deficient Lentiviral Vectors: Certain lentiviral vectors are designed to be less pathogenic than wild-type lentiviruses due in part to the separation of genes required for packaging of viral particles onto several plasmids, replacement of the native lentiviral envelope protein, and elimination of accessory genes that are essential for replication of wild-type lentiviruses. Lentiviral vector systems designed with these enhanced safety features are not able to replicate in human cells and are defined as replication-deficient lentiviral vectors.

2.3 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 are at risk.

2.4 Laboratory Hazards: Penetration through the skin via puncture, cut, or absorption through broken skin (*e.g.* scratches, cuts, abrasions, dermatitis, or other lesions) and/or mucous membrane exposure via splash to the eyes, nose, and/or mouth are known to be potential exposure pathways for lentiviral agents.

3. REGISTRATION REQUIREMENTS:

All Principal Investigators (PIs) working with clinical specimens containing blood, blood components, body fluids and/or tissues from humans, cultures of lentiviruses or lentiviral vectors, or animals or unfixed specimens from animals infected with lentiviruses or lentiviral vectors must be registered with Environmental Health & Safety (EH&S).

3.1 Use in Animals: Registration is accomplished via completion of an electronic IACUC protocol for work with animals involving lentiviruses or lentiviral vectors.

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3.2 Use *in vitro* and/or Use of Recombinant or Synthetic Nucleic Acids: Investigators performing solely *in vitro* experiments, should complete a registration document obtained from www.ehs.pitt.edu/biological/workbook.html. PIs working with recombinant or synthetic nucleic acids in lentiviral vectors or performing recombinant or synthetic techniques using genetic material from lentiviruses must be registered with the Institutional Biosafety Committee. IBC application documents may be obtained from <http://www.ibc.pitt.edu/Apply/apply.htm>.

4. ASSIGNING BIOSAFETY LEVELS FOR WORK WITH LENTIVIRUSES

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 with lentiviruses and lentiviral vectors. For work with lentiviruses, biosafety level assignment is based on the research-specific risks (*e.g.* sample population, specific hazards associated with techniques), pathogenicity of the lentivirus in use, and design of laboratory facilities. **Final biosafety level determination for work with lentiviruses and lentiviral vectors containing recombinant or synthetic nucleic acids will be made by the Institutional Biosafety (IBC) Committee.** The University Biohazards Committee will provide guidance as necessary, and must be consulted for all work being considered at the BSL-3 level.

4.1.1 Biosafety Level 2 (BSL-2): BSL-2 is appropriate for:

4.1.1.1 Work with diagnostic specimens that contain human blood, body fluids or tissues,

4.1.1.2 Generating and using IBC-approved, replication-deficient lentiviral vectors,

4.1.1.3 Work with lentiviruses or lentiviral vectors based on lentiviruses such as FIV and EIAV that are not known to be infectious to humans, and

4.1.1.4 Handling animals, animal tissues, blood, body fluids and/or cell lines infected with replication-deficient lentiviral vectors, that **do not** express oncogenes, if the vectors meet all other criteria listed in Table 1 below.

4.1.2 Biosafety Level 2+ (BSL-2+): At the University of Pittsburgh, there is a specialized biosafety level defined as BSL-2+. In BSL-2+ laboratories, additional work practices and containment equipment described in biosafety guidelines for work at BSL-3 are used in a BSL-2 facility (additional detail is provided below in Section 4.2). BSL-2+ is appropriate for:

4.1.2.1 Processes that include culture and production of known or potentially human pathogenic lentiviruses (*e.g.* HIV, SIV, recombinant forms of HIV/SIV/SHIV),

4.1.2.2 Manipulation of human pathogenic lentivirus-infected samples for research purposes,

4.1.2.3 Use of lentiviral vectors that contain oncogenes or that are not replication deficient (see Table 1),

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4.1.2.4 Manipulation of high-titer virus preparations in volumes ≥ 100 mL but ≤ 10 L, and/or,

4.1.2.5 Procedures with a high likelihood of droplet or aerosol formation.

4.1.3 Biosafety Level 3 (BSL-3): Activities involving large scale preparation (≥ 10 Liters) of concentrated, high-titer lentiviruses shall be conducted in an approved BSL-3 facility, using BSL-3 practices and containment equipment. Any BSL-3 work must be approved by the University Biohazards Committee and EH&S. Additional approval from the IBC is required for work with large scale volumes of lentiviral vectors modified with recombinant and/or synthetic nucleic acids.

4.1.4 Animal Housing: Animals infected with lentiviruses and/or lentiviral vectors shall be housed in ABSL-2 facilities at a minimum. The IBC may assign work with animals to ABSL-2 or ABSL-3 depending on specific hazards and risks of the project, including but not limited to, animal species, specific agent, and experimental manipulations.

4.2 Definition of BSL-2+ at the University of Pittsburgh: Following completion of registration, laboratories are inspected by EH&S to verify appropriate containment and work practices. The criteria for BSL-2+ includes meeting all facility containment requirements for BSL-2, while following specific BSL-3 work practice requirements, as outlined in *CDC Biosafety in Microbiological and Biomedical Laboratories*, 5th edition, and the *NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules*, latest edition.

The specific additional practice requirements for BSL-2+, in addition to all standard work practices required at BSL-2, shall, at a minimum, include the following:

4.2.1 An investigator-specific BSL-2+ Biosafety Operations Manual must be prepared by the investigator, and must include:

4.2.1.1 An approval page signed by the PI and the Biosafety Officer, and the animal facility director if work with lentiviruses or lentiviral vectors involves animals,

4.2.1.2 Emergency contact numbers for laboratory management personnel,

4.2.1.3 Agents to be used, locations of use, and details regarding safe, access-controlled storage of agents,

4.2.1.4 Procedures describing proper response and clean-up of spills of lentiviral cultures, infected cell cultures, and other potentially infectious material,

4.2.1.5 Procedures describing proper first aid and medical response procedures for personnel who may be exposed to lentiviral cultures, infected materials or animals,

4.2.1.6 Laboratory-specific training requirements for personnel who will work with lentivirus cultures, and/or infected cells and animals as well as records demonstrating completion of this training,

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4.2.1.7 Laboratory-specific standard operating procedures for routine laboratory tasks, including safe handling of infectious agents, required facility-specific personal protective equipment (PPE), decontamination and disposal of waste, and

4.1.2.8 Autoclave verification program, if autoclave is used for decontamination.

4.2.2 Access to laboratory must be restricted to personnel trained in the contents of the Biosafety Operations Manual while work with lentiviruses or lentiviral vectors is in progress.

4.2.3 The laboratory shall be negatively pressurized to surrounding spaces.

4.2.4 The Principal Investigator is responsible for ensuring that all personnel *demonstrate proficiency* in the practices and operations of the facility prior to beginning unsupervised work at BSL-2+.

4.2.5 All vacuum lines used to aspirate infected cultures must be protected with liquid disinfectant traps and in-line HEPA filters (BMBL 5th Edition, Appendix A, Figure 12).

4.2.6 A certified Biological Safety Cabinet (BSC) *must* be used for all manipulations involving infectious materials.

4.2.7 Centrifuge safety cups and/or safety rotors *must* be used for centrifugation outside of a BSC. Safety cups and safety rotors must not be opened for loading/unloading of samples outside of a certified BSC.

4.2.8 Personal protective equipment must consist of the following and must be worn at all times within the BSL-2+ facility:

4.2.8.1 Either 1) a disposable, liquid-barrier wrap around gown, or 2) a standard, BSL-2+ facility-dedicated button front lab coat with a liquid-barrier wrap around apron and disposable sleeve covers;

4.2.8.2 Mucous membrane splash protection consisting of a full face shield or safety glasses, in combination with a surgical mask for anticipated splashes or sprays of infectious materials.

4.2.8.3 Double gloves (latex over nitrile or nitrile over nitrile);

4.2.9 All personal protective equipment must be removed and either properly decontaminated and stored, or disposed of prior to leaving the laboratory.

4.2.10 All potentially contaminated solids and/or liquids must be properly decontaminated prior to removal from the facility (*e.g.* soaked in bleach and disposed of in infectious waste stream; surface decontaminated with an appropriate EPA-registered disinfectant; double-bagged and autoclaved; liquids decontaminated with appropriate EPA-registered disinfectant prior to sink disposal)

4.2.11 Animal cages must be autoclaved before they are cleaned and washed.

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4.2.12 Personnel must be notified that serum surveillance is available to all individuals potentially exposed to lentiviruses or lentiviral vectors. See EH&S Guideline 05-11: Serum Surveillance Program (<http://www.ehs.pitt.edu/assets/docs/serum-surveillance.pdf>).

5. ADDITIONAL GUIDANCE FOR ASSIGNING BIOSAFETY LEVELS FOR WORK WITH LENTIVIRAL VECTORS

5.1 Biosafety Level Assignment Guidance: Biosafety considerations are based on the specific lentivirus or lentiviral vector system used, the potential for oncogenic activity of expressed genes, and scale of production of the lentivirus or lentiviral vector. **Final biosafety level determination will be made by the Institutional Biosafety Committee.** The University Biohazards Committee must be consulted for any work being proposed for BSL-3/ABSL-3.

5.2 Expression of Oncogenes or Genes with Oncogenic Activity in Lentiviral Vectors: All lentiviral vectors expressing oncogenes or genes with oncogenic activity must be handled at BSL-2+, regardless of the packaging system used.

5.3 Production of Concentrated Lentiviral Preparations: Investigators planning to produce quantities of concentrated, high-titer lentiviral vectors by preparing 100 mL or more of culture, but not exceeding 10 L, must produce lentiviral preparations at BSL-2+ regardless of packaging system used.

5.4 3rd Generation, 4-Plasmid (or more) Lentiviral Systems: It is strongly recommended that investigators use 3rd generation, 4-plasmid lentiviral vector systems available from commercial vendors, recognized vector cores, or other investigators at the University of Pittsburgh. The IBC does not require testing for replication competent viruses (RCV) for generation and/or use of small volumes (≤ 100 mL) of 4-plasmid (3rd generation) systems that do not express genes with oncogenic activity at BSL-2/ABSL-2 (Table 1).

5.5 2nd Generation, 3-Plasmid (or less) Lentiviral Systems. 2nd generation, 3-plasmid lentivirus systems must be generated and used at BSL-2+. Investigators may request to conduct such research at BSL-2 following demonstration that virus preparations have no detectable RCV. If this data is not available at the time of original IBC protocol submission, an IBC protocol modification requesting BSL-2 conduct must be submitted. The modification must detail the type of testing used to ensure that no detectable RCV is produced and the data from the RCV testing if performed by the investigator (see section 4.6 below).

IBC approval of the modification must be received in writing before any 2nd generation, 3-plasmid lentiviral systems may be handled at BSL-2 (Table 1).

Approval for BSL-2 handling of 2nd generation, 3-plasmid systems at BSL-2 shall be specific to each virus preparation made. If separate preparations expressing different recombinant or synthetic nucleic acids are generated, each preparation must be demonstrated to be free of RCV and approved by the IBC prior to handling at BSL-2.

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Table 1. Summary of biosafety level requirements for lentiviral 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	Not 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+

5.6 Replication Competent Virus Assay: Testing for RCV may be performed by individual investigators using a standard p24 ELISA kit, provided that the assay has a sensitivity of ≤ 12.5 pg/ml.

A positive control for virus infection is not required; neither the IBC nor EH&S 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.

The Magee-Women's Research Institute Transgenic and Molecular Core (Director: Kyle Orwig; Administrator: Jennifer Orwig; phone 412 641-2415) is available for a fee to perform the RCV assay for individual investigators. If the MWRI Transgenic and Molecular Core performs the assay for the investigator, the p24 ELISA assay report provided by the Core must be submitted to the IBC office.

5.5 Exceptions to the requirement for RCV testing of 3-plasmid lentivirus stock. Investigators who acquire prepared lentiviral vector stocks from a commercial source that provides documentation of acceptable RCV testing will not be required to test for RCV. Manufacturer-specific RCV testing 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 prepared lentiviral vector stocks from a University of Pittsburgh investigator, an established University Vector Core laboratory, or an investigator from another institution should contact the IBC regarding requirements for RCV testing, which will be reviewed on a case-by-case basis.

6. REFERENCES

6.1 Biosafety in Microbiological and Biomedical Laboratories. 5th edition. Washington, D.C. Centers for Disease Control and Prevention and National Institutes of Health; December, 2009.

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6.2 NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules. Bethesda. The National Institutes of Health Office of Biotechnology Activities; November, 2014.

6.3 Biosafety Considerations for Research with Lentiviral Vectors: Recombinant DNA Advisory Committee (RAC) Guidance Document. The National Institutes of Health Office of Biotechnology Activities. http://osp.od.nih.gov/sites/default/files/resources/Lenti_Containment_Guidance_0_0.pdf; Accessed 06/26/2014.

6.4 University of Pittsburgh Institutional Biosafety Committee Guidance on Biosafety Level Assignment for Lentiviral Vectors. University of Pittsburgh IBC. <http://www.abc.pitt.edu/ViralVector/ViralVector.htm>; Accessed 06/26/2014.