# Research with Lentivirus and Lentiviral Vector Systems

**Policy #S-002**

VA Pittsburgh Healthcare System
Pittsburgh, PA 15240

**Signatory Authority:**Dr. Steven Graham, ACOS

**Responsible Owner:**Associate Chief of Staff/R&D

**Service Line(s):**Research and Development (R&D) Department

**Effective Date:**June 16, 2020

**Recertification Date:**December 2021

## PURPOSE AND AUTHORITY

* 1. To provide biosafety considerations and appropriate work practices when working with human pathogenic lentiviruses and lentiviral vector systems at the VA Pittsburgh Healthcare System (VAPHS). The policy contains information regarding working with human pathogenic lentiviruses as well instructions for use of lentiviral vectors. Recommendations from the *CDC Biosafety in Microbiological and Biomedical Laboratories*, 5th edition, the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*, April 2016, and the NIH Recombinant DNA Advisory Committee guidance of October 2006 are incorporated into this policy.
	2. This SOP sets forth mandatory procedures and processes to ensure compliance with VHA Directive 1200.08, Safety of Personnel and Security of Laboratories Involved in VA Research, April 24, 2019.
	3. This policy applies to all laboratory research conducted at or under the auspices of the VAPHS.

## PROCEDURES for WORKING WITH LENTIVIRUSES/LENTIVIRAL VECTORS

* 1. Biosafety Level Assignment and Work Practice Guidance

The following designations are provided to assist in the determination of the appropriate biosafety level and work practices to be used when working with lentivirus and lentiviral vectors. Biosafety level assignment is based on the research-specific risks associated with the project (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 VAPHS Institutional Biosafety Committee (IBC)**.

1. Biosafety Level 2 (BSL-2) is appropriate for the following:
* Diagnostic specimens that contain human blood, body fluids, or tissues
* Generating and using IBC-approved replication-deficient lentiviral vectors
* Handling animals and animal tissues, blood, body fluids, and cell lines from animals infected with replication-deficient lentiviral vectors as long as they **do not** express oncogenes and meet other criteria listed in Table 1 below
* Work with lentiviruses or lentiviral vectors based on lentiviruses such as FIV and EIAV that are not infectious to humans can be performed at BSL-2
1. Biosafety Level 2+ (BSL-2+) - This designation was developed to allow work with slightly more hazardous organisms and procedures in a BSL-2 environment using enhanced procedures and work practices that exceed standard BSL-2 requirements, including appropriate safety equipment (biosafety cabinets, safety centrifuge cups, etc.). BSL-2+ if appropriate for the following:
* Processes that include culture and production of known or potentially human pathogenic lentiviruses (such as HIV, SIV and recombinant forms of HIV/SIV/SHIV)
* Manipulation of human pathogenic lentivirus-infected samples for research purposes
* Use of lentiviral vectors that express oncogenes or that are not replication deficient (see Table 1),
* Manipulation of high titer virus preparations in volumes greater than or equal to 100 milliliters but less than 10 liters
* Procedures with a high likelihood of droplet or aerosol formation
1. Animal Housing - Animals infected with lentiviruses and/or lentiviral vectors are housed in ABSL-2 facilities. The IBC may assign work with animals to ABSL-2+ depending on specific hazards and risks of the project, including but not limited to animal species, specific agent, and experimental manipulations.
2. Research designated as BSL-3 does not occur at the VAPHS since there are no laboratories able to support BSL-3 activities.
	1. Laboratory Inspection Requirements

Laboratories are inspected to verify appropriate containment and 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 the *CDC Biosafety in Microbiological and Biomedical Laboratories*, 5th edition, and the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules,* April 2016. 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:

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

Approval page signed by the Principal Investigator and the Biosafety Officer, and the Animal Research Facility Supervisor if work with lentiviruses or lentiviral vectors involves animals;

Emergency contact numbers for laboratory management personnel;

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

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

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

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

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

Autoclave verification program, if autoclave is used for decontamination.

1. 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.
2. The laboratory airflow must be negative when compared to surrounding spaces.
3. 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. All vacuum lines used to aspirate infected cultures must be protected with liquid disinfectant traps and in-line HEPA filters.
5. A certified Biological Safety Cabinet (BSC) *must* be used for all manipulations involving infectious materials.
6. Centrifuge safety cups and/or safety rotors *must* be used for centrifugation outside of a BSC. Safety cups or safety rotors must only be opened within a certified BSC.
7. PPE must consist of the following and must be worn at all times within the BSL-2+ facility:
* Either a disposable, liquid-barrier wrap around gown or a standard BSL-2+ facility-dedicated button front lab coat with liquid-barrier wrap around apron and disposable sleeve covers
* 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
* Two pairs of gloves (latex over nitrile or two pairs of nitrile)
1. All PPE must be removed and either properly decontaminated and stored or disposed of prior to leaving the laboratory.
2. 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 the infectious waste stream; surface decontaminated with appropriate EPA-registered disinfectant; double bagged and autoclaved; liquids decontaminated with appropriate EPA-registered disinfectant for appropriate contact time prior to sink disposal).
3. Animal cages must be autoclaved or decontaminated before they are cleaned and washed.
4. Personnel must be notified that serum surveillance is available to all individuals potentially exposed to lentiviruses or lentiviral vectors (see section 7.0 below).
	1. Assigning Biosafety Levels for Work with Lentivirus/Lentiviral Vectors
5. 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. Guidance and restrictions for lentiviral vector work is described below and summarized in Table 1. **Final biosafety determination will be made by the VAPHS IBC**.

1. 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.

1. Production of Concentrated Lentiviral Preparations

 Investigators planning to produce quantities of concentrated, high-titer lentiviral vectors by preparing 100 milliliters or more of culture, but not exceeding 10 liters, must produce lentiviral preparations at BSL-2+ regardless of the packaging system used.

1. 3rd Generation, 4-Plasmid (or More) Lentiviral Systems

It is strongly recommended that investigator’s use 3rd generation, 4-plasmid lentiviral vectors systems available from commercial vendors, recognized vector cores, or collaborators. The IBC does not require testing for replication competent viruses (RCV) for generation and/or use of small volumes (less than 100 milliliters) of 4-plasmid (3rd generation) systems that do not express genes with oncogenic activity at BSL-2/ABSL-2 (see Table 1).

1. 2nd Generation, 3-Plasmid (or Less) Lentiviral Systems

2nd generation, 3-plasmid lentivirus systems must 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 requesting BSL-2 designation must be submitted and include the name of the RCV test used and all data from the RCV test (refer to Section E). If this data is not available at the time of the 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 6.4 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.

Approval for BSL-2 use with 2nd generation, 3-plasmid systems will be specific to each virus preparation made. If separate lentivirus 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.

**TABLE 1. Summary of biosafety level requirements for lentiviral vector production and use**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Oncogenic transgene or >100 ml production** | **Number of Plasmids** | **RCV Testing** | **Vector Production** | **Use of Viral Vectors in vitro** | **Use of Viral Vectors in vivo** | **Use of virus-transfected cells in vivo** |
| 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 |
| No | 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 | 3 | No RCV test | BSL-2+ | BSL-2+ | ABSL-2+ | ABSL-2+ |

1. Replication Competent Virus Testing

Testing for RCV can 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; the IBC does not want the investigator to work with infectious lentivirus 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 three (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 IBC Coordinator (Elizabeth.Toth2@va.gov).

1. 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 another investigator, an established Vector Core Facility, or an investigator from another institution should contact the IBC Coordinator regarding requirements for RCV testing, which will be reviewed on a case-by-case basis.

* 1. Serum Surveillance

Serum testing will be available to individuals with potential exposure to lentiviruses, per the VAPHS MCM IC-004 Human Immunodeficiency Virus (HIV) Diagnostic Testing. See the memorandum for additional information on this program.

## ASSIGNMENT OF RESPONSIBILITIES

* 1. Final biosafety level determination for work with lentiviruses and lentiviral vectors containing recombinant or synthetic nucleic acids will be made by the VAPHS Institutional Biosafety Committee (IBC).

## DEFINITIONS

* 1. **Lentivirus** - 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) and simian immunodeficiency virus (SIV) that 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. **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.
	3. **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.
	4. **Employees Potentially at Risk** - Laboratory personnel that handle pathogenic lentiviruses, recombinant lentiviral vectors, naturally or experimentally infected laboratory animals, or clinical specimens potentially infected with lentivirus are at risk.
	5. **Body Entry Routes** -Penetration through the skin via puncture 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 considered potential exposure pathways for lentiviral agents.

## REFERENCES

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

-NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules. Bethesda. The National Institutes of Health Office of Biotechnology Activities; April 2016.

-Biosafety Considerations for Research with Lentiviral Vectors: Recombinant DNA Advisory Committee (RAC) Guidance Document. The National Institutes of Health, Office of Biotechnology Activities.

-VHA Directive 1200.08, Safety of Personnel and Security of Laboratories Involved in VA Research, April 24, 2019, <https://www.research.va.gov/resources/policies/handbooks.cfm>

## REVIEW

This policy is reviewed at recertification, when there are changes to the governing document (for example, national policy or an accreditation body mandate), and any regulatory requirement for more frequent review.

## RECERTIFICATION

This Policy is scheduled for recertification fifteen (15) months from the Effective Date. In the event of contradiction with national policy, the national policy supersedes and controls.

## SIGNATORY AUTHORITY

*//signed copy on file //*

Gretchen Haas, MD

Chair, Research and Development Committee

Date Approved: June 16, 2020

*//signed copy on file //*

Steven Graham, MD, PhD

Associate Chief of Staff Research and Development Department

Date Approved: June 16, 2020

***NOTE:*** *The signature remains valid until rescinded by an appropriate administrative action.*

**DISTRIBUTION:** Policy is available at: <https://www.va.gov/pittsburgh-health-care/research/safety-security/>