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# Herpes Simplex Virus Vectors

## Background

Herpes Simplex Virus 1 and 2 (HSV-1 and HSV-2) are enveloped viruses belonging to the family Herpesviridae, composed of a linear, double-stranded DNA genome. Both HSV-1 and HSV-2 are pathogenic in humans and are life-long infections. HSV has been used as a viral vector therapy for nervous system disorders due to its natural tropism for neuronal cells. HSV vectors have a wide host range and cell tropism, and can infect almost every vertebrate cell type. Typically, HSV vectors are replication deficient due to deletions in the viral genome. These vectors have an unparalleled transgene capacity (up to 150 kbp) and can have life-long persistence in neurons without integration into the genome. Despite the majority being replication-deficient vectors, recombination into a replication-competent virus could occur if the vector infects a cell with a pre-existing herpes virus infection.

There are two main types of HSV vectors: replication-defective HSV-1 and HSV-1 amplicon vectors. Amplicon vectors relies on helper virus functions for their production, which provides the possibility for helper virus contamination, cytotoxicity, and immunogenecity. Replication defective vectors are still infectious and can induce potent inflammatory responses. Replication competent viruses can establish transient infection in humans, but not latent infection in neurons.

## Symptoms of Exposure

HSV infection classically results in fever blisters or cold sores on either the oral mucosa (HSV-1) or the genital areas (HSV-2). However, both HSV-1 and -2 can infect the oral mucosa and genital region.

## Environmental Stability

Enveloped viruses are rapidly inactivated when exposed to drying environments. However, it remains prudent practice in BSL-2 to disinfect with a broad-spectrum disinfectant, such as sodium hypochlorite (i.e. 1:10 bleach solution). This solution must be made daily.

## Modes of Transmission

HSV-1 is typically transmitted by saliva or by the infection on hands of healthcare personnel. HSV-2 is typically transmitted through sexual contact. HSV can be transmitted by direct contact with epithelial or mucosal surfaces. In the laboratory, HSV can be transmitted by ingestion, parenteral injection, droplet exposure of the mucous membranes (eyes, nose or mouth), and inhalation of aerosolized materials.

## Host Range

HSV infects a wide range of vertebrate hosts and a wide variety of cell types.

## Approvals

Experiments using HSV require IBC approvals before initiation of experiments.

## Test Methods for Replication Competent Virus

**If vectors are being obtained from a commercial supplier, please check the manufacturer's information as to the quality control concerning replication competent viruses. This information should be supplied with the IBC application.

Viral preparations used for *in vitro* studies should be tested every 6 months for replication competent viruses by plaque assay. These assays should be tested at a sensitivity limit of 1 infectious unit per mL. Viral preparations used in animals should be tested for replication competent viruses before each use by plaque assay (Strathdee, 2000).

## Laboratory Practices

Generally, HSV is classified as a **Biosafety Level 2** (BSL-2) organism requiring BSL2 practices and procedures for all virus and Animal Biosafety Level – 2 (ABSL-2) for all animal manipulation as well as animal housing. At the discretion of the IBC, experiments may need to be conducted at Biosafety Level - 3 (BSL3). In the IBC application, the PI must justify that the gene to be expressed is not particularly harmful, and include citations to support these statements.

1. No work with HSV or HSV vectors is permitted on the open bench.

2. A certified Class II biosafety cabinet must be used for all manipulations including (but not limited to):
   - Pipetting
   - Harvesting infected cells for RNA
   - Purification of virus
   - Infection of cell culture
   - Infection of animals

3. Centrifugation must be done in closed containers with **sealed rotors or safety cups**. Safety cups are to be loaded and unloaded inside the biosafety cabinet.

4. All vacuum lines must be fitted with a HEPA filter (an example is the "Vacushield ™" inline hydrophobic filter, Product # 4402 from Gelman Science , Millex FH vacuum line protector Millipore (Fisher) cat # SLFH05010, or "HEPA-VENT™" inline hydrophobic filter, Catalog # 6723-5000 from Whatman).

5. All laboratory staff working with or supervising work with herpes viruses must be made aware of the hazards associated with the work, required safety practices and procedures, and proper handling of the agent, as well as be current on required laboratory safety and biosafety trainings.

6. Animal carcasses must be placed in autoclave bags and be designated for infectious waste disposal.

7. Special training must be given to all animal husbandry personnel on retroviruses, the hazards associated with the work, required practices and procedures and proper handling of bedding, cage washing, and all other husbandry materials associated with the experiment. This training would be provided by animal facility supervisors in consultation with REHS.

8. Signs and labels (universal biohazard symbol) must be placed to indicate each area where herpes viruses are used or stored (including biosafety cabinets, incubators, refrigerators, laboratory

entrance doors, etc.) The signs should include the name of the agent, emergency contact information and a biohazard sticker.

9. All work and manipulations of retrovirus must be conducted in a certified Class II biological safety cabinet. If there are extenuating circumstances or a biosafety cabinet is unavailable, please contact REHS (at the numbers listed at the end of this SOP) as additional precautions may be required.

## Personal Protective Equipment

1. Disposable gloves.

2. Disposable gown or equivalent when introducing vector into animals or performing necropsies. Lab coats are adequate for tissue culture manipulations.

3. Eye Protection.

## Instructions in the Event of Employee Exposure

- **EXPOSURE FROM SPLASH OR AEROSOLS – INHALATION**

  Report the incident to your supervisor and refer to the Rutgers Emergency Action Plan for further instructions. The supervisor should submit an incident report through https://MyREHS.rutgers.edu to document the event.

- **EXPOSURE FROM SPLASH OR AEROSOLS – EYE CONTACT, SKIN AND/OR MUCOUS MEMBRANE**

  Rinse a minimum of 15 minutes in eye wash or flush area with water, report the incident to your supervisor and refer to the Rutgers Emergency Action Plan posted in the lab for further instructions. The supervisor should submit an incident report through https://MyREHS.rutgers.edu to document the event.

- **NEEDLESTICK AND/OR SHARPS EXPOSURE**

  Contaminated skin should be thoroughly scrubbed for several minutes with soap or a 10% povidone solution (Betadine) and copious amounts of water. Report the incident to your supervisor and REHS immediately after scrub. Seek medical attention at Campus Employee Health Services/Occupational Medicine Services. Refer to Rutgers Emergency Action Plan posted in the lab for after-hours exposure. The supervisor should submit an incident report through https://MyREHS.rutgers.edu to document the event.

- **EMERGENT EXPOSURES**

  For situations in which exposure to AAV occurred and medical treatment is an emergency, personnel should report to the Emergency Room, and ensure their supervisor completes incident report through https://MyREHS.rutgers.edu to document the event.

## Decontamination

The most effective disinfectant against HSV is a 1:10 Sodium hypochlorite (bleach) solution that is made fresh daily.

- To make this solution, dilute 1 part bleach to 9 parts tap water.
- Ensure a 15 minute contact time.
- Use this disinfectant for treatment of reusable equipment, surfaces, and liquid waste (final volume 1% bleach).

Disinfectant alternatives include phenolics, 2% glutaraldehyde, and 70% ethanol.

Autoclaving for 1 hour at 121°C or 250°F (15 lbs psi of steam pressure).

- Use this disinfection method for reusable equipment, liquid waste or solid waste.

## Animal Practices

1. When animals are infected with herpesvirus vectors, an Animal Biosafety Level – 2 (ABSL-2) area must be approved and used for the procedure. Concurrent approvals are needed from the Institutional Biosafety Committee (IBC) and the Institutional Animal Care and Use Committee (IACUC).

2. All bedding, waste and animals shall be treated as biohazardous. Cage changing and husbandry must be performed according to the hazard sign provided by REHS. All waste must be decontaminated by autoclaving or chemical disinfection prior to disposal.

3. Animal carcasses must be placed in autoclave bags and be designated for infectious waste disposal.

4. All necropsies must be performed in a designated room using animal BSL-2 practices and procedures.

5. The following information must be posted on the door of the animal room. REHS will provide a sign template to the animal facility staff for this purpose.

    - A description of special housing required to ensure safety of animal facility personnel, such as ventilated cabinets or hoods.
    - A label on the animal cage indicating the hazardous materials to be administered to live animals. (i.e., HSV-1)
    - The name of individual(s) responsible for handling the materials**.** (i.e., Drs. X, Y and Z and Technicians A and B as per protocol #00000)
    - A description of how to handle animals, carcasses, and contaminated cages and bedding

## References


- CDC-BMBL, 5th ed., www.cdc.gov/od/ohs/biosfty/bmbl5/BMBL_5th_Edition.pdf

- Stanford University, “Working with Viral Vectors,” https://ehs.stanford.edu/topic/biosafety-biosecurity/viral-vectors

- Strathdee CA, McLeod, MR. 2000. “A modular set of helper dependent simplex virus expression vectors.” Mol Ther. 5: 479-485.

- Young, L.S., Searle, P.F., Onion, D., and V. Mautner. 2006. “Viral gene therapy strategies: from basic science to clinical application.” J. of Pathology. 208:299-318.

- Braun, A. 2006. “Biosafety in Handling Gene Transfer Vectors.” Current Protocols in Human Genetics. 12.1-12.18.

# Standard Operating Procedures

# Acknowledgement Page

I, _____, have read the SOP for working with _____ Viral Vector. The following people will be conducting experiments using these vectors. The staff members know where to find a copy of this SOP in the laboratory and they understand the hazards and safe work practices as detailed therein.

| **Name** | **Job Title** | **Initials** |
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Principal Investigator (print): _____

Principal Investigator (Signature):_____