STANDARD ◆ OPERATING ◆ PROCEDURES

Herpes Simplex Virus Vectors

Background

Herpes Simplex Virus (HSV) is a DNA virus with two serological types, HSV-1 and -2. HSV vectors have a wide host range and cell tropism, and can infect almost every vertebrate cell type. The virus can establish latent infection in post mitotic neurons, which allows it to be a useful tool in transducing cells in the nervous system. Typically, HSV vectors are replication deficient due to deletions in the viral genome

Symptoms of Exposure

Herpes Virus infections 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.

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 local IBC approvals before initiation of experiments.

Test Methods for Recombinant Virus-QC Tests

**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 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
   - Infection of cell culture
   - Infection of animals

3. Centrifugation must be done in closed containers and using **sealed rotors or safety cups**. Safety cups are to be opened 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 HSV 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 training requirements.

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 replication competent herpesvirus, 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. Animal facility staff may provide this training in consultation with REHS.

8. Signs and labels (universal biohazard symbol) must be placed to indicate each area where HSV is used or stored (including biosafety cabinets, incubators, refrigerators, laboratory entrance doors, etc.)

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. Goggles and/or face shield.

4. All work and manipulations of HSV 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.

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 Emergency Response Guide flip chart 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 a 10% povidone solution (Betadine) and copious amounts of water. Report the incident to your supervisor and EOHSS immediately after scrub. Seek medical attention at Campus Employee Health Services/Occupational Medicine Services. Refer to Emergency Response Guide flip chart 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% Sodium hypochlorite (bleach) solution that is made fresh daily.

- To make this solution, dilute 1 part Clorox to 5 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. Rodents must be in closed caging i.e. with filter-top bonnets or ventilated caging and must have enough food and water after adenoviral administration so that husbandry and investigatory staff need not open a cage for 72 hours. For example, only as much as 400-grams or less of rats (up to one 400-gram rat or four 100-gram rats) can be permitted in a standard rat cage without a cage change for 72 hours).

3. All bedding, waste and animals infected with HSV shall be treated as biohazardous. After all animals are removed from their primary enclosure immediately autoclave or treat with chemical disinfectant. After disinfection, dump the cage contents and begin cleaning the cage for re-use. All waste must be decontaminated by autoclaving or chemical disinfection prior to disposal.

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

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

6. The following information must be posted in 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 the hazardous materials are to be used in the protocol. See the following example: *"The protocol will be conducted at Biosafety Level 2 (BSL2) and Animal Biosafety Level 2 (ABSL2). Staff will use Class II biosafety cabinets for procedures that have the potential to generate aerosols, splash or spray. Personnel working on the protocol will be trained in the hazards of working with the recombinant herpesvirus vectors and the measures they need to take to protect themselves. They will wear lab coats or gowns, protective gloves and protective eyewear for all aspects of the protocol."*

References

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

Stanford University, "Working with Viral Vectors," http://www.stanford.edu/dept/EHS/prod/researchlab/bio/docs/Working_with_Viral_Vectors.pdf

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.

Herpes Simplex Virus Vectors: Standard Operating Procedures

Acknowledgement Page

I, _____, have read the SOP for Herpes Simplex Virus Vectors. The following people will be conducting experiments using the HSV 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

Principal Investigator (print): _____

Principal Investigator (Signature):_____

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