

Tel-Aviv University –Safety Unit

Standard Operating Procedure for Working with *Herpes simplex virus (HSV)* in Animals.

1. Health hazards

Herpes simplex viruses (HSV) are human pathogens represented by two distinct serotypes: HSV-1 and HSV-2 (also known as human herpesvirus 1 and 2 (HHV-1 and HHV-2)), belong to the sub family Alphaherpesviridae in the family Herpesviridae, genus Simplexvirus. They are 120-300 nm in diameter and consist of a linear, double stranded DNA genome, enclosed within an icosahedral capsid, surrounded by a phospholipid rich envelope. The lipid envelope is derived from the nuclear envelope of the infected cell.

These viruses cause significant morbidity, primarily as mucosal membrane lesions in the form of facial "cold sores" or "fever blisters" and genital ulcers. HSV establish latent infection in dorsal-root ganglia for the entire life of the host. From this reservoir, they can reactivate, to cause human morbidity and mortality.

HSE (herpes simplex virus encephalitis) an infections of the CNS, is one of the most devastating disorders caused by these viruses. It is not clear yet, whether HSE is the result of primary infection or is it the outcome of reactivation.

Other diseases caused by HSV:

Herpetic whitlow: Characterized by formation of painful vesicular lesions on the nail or finger area.

Infections of the eye: Characteristic dendritic ulceration occurs on conjunctiva, and cornea.

HSV infection may cause other ocular diseases, including blepharitis/dermatitis, conjunctivitis, dendritic epithelial keratitis, corneal ulceration and Neonatal herpes, which is an extremely severe disease with a very high mortality rate.

It was reported in 1979, that there is a possible link between HSV-1 and Alzheimer's disease, in people with the epsilon4 allele of the gene APOE.

MODE OF TRANSMISSION:

Direct contact with infected secretions or mucous membranes/skin with lesions from an asymptomatic or symptomatic patients shedding the virus, is the main mode of transmission of HSV. **Transmission of HSV-1 can also occur by respiratory droplets.**

	<p>Genital herpes is transmitted sexually and Neonatal herpes can be acquired at different times: intrauterine (in utero) in 5% of the cases, peripartum (perinatal) in 85% of the cases, and postpartum (postnatal) in 10% of the cases.</p> <p>HOST RANGE: Humans, but non-human primates in captivity can be accidentally infected. Rabbits and rodents can be infected experimentally.</p> <p>ZOOZONOSIS: None.</p> <p>VECTORS: None.</p>
2. Designated Area	ABSL-2 facility.
3. Training	Practical experience with animal care/maintenance, as well as general biosafety, is required.
4. Personal Protective Equipment (PPE)	Gloves, Eyes safety goggles, Lab coat, Disposable shoe covers and Animal handling gown.
5. General Precautions for Animal Use	<p>Inhalation of virus from aerosols, generated when aspirating, dispensing, or mixing virus-infected samples (tissues, feces, secretions) from infected animals. Laboratory infection can also occur from direct contact with clinical material or viral isolates, inhalation of concentrated aerosolized materials, droplet exposure of mucous membranes of the eyes, nose, or mouth, ingestion, accidental parenteral inoculation are the primary hazards associated with herpes viruses including HSV 1 and 2.</p> <p>Inoculation of mucous membranes via virus contaminated gloves following the handling of tissues, feces and/or secretions from infected animals.</p>
6. Environmental / Ventilation Controls	<p>Work should be conducted in ABSL-2 facility, over absorbent pads in a class II type A1 or A2 biological cabinet.</p> <p>SURVIVAL OUTSIDE HOST: HSV virus survives for short periods of time outside the host. It can survive on dry inanimate surfaces (survival ranges from few hours to 8 weeks). They survive longer at lower humidity.</p>

7. Animal handling practices

1. Animals must be housed in filter top cages marked as biohazards (including the name of the pathogen/biohazard). Handling the cages (including bedding) will be done only by the researchers.
2. Use a class II Biological Safety Cabinet at all times (especially during injection or any surgical procedure), when performing work on these animals and/or when moving animals from dirty to clean cages.
3. Infected animals may shed HSV after treatment; take precautions to avoid the creation of aerosols when changing or washing cages, or cleaning the room.
4. Dead animals must be placed in primary plastic bags, which are then placed in biosafety bags for infectious waste incineration.
5. All surfaces and racks that may be contaminated will be decontaminated with 0.5% bleach (or virusolve), ASAP.

6. When changing cages, use a standard microisolator technique:

- Place the cage containing the animals, under the biological safety cabinet and transfer the animals into a clean cage.
- Spray the dirty cage with 0.5% bleach (or virusolve), remove from the safety cabinet and place on a transfer rack .
- When all cages have been changed, spray the dirty cages and rack again with 0.5% bleach, and cover the rack. Put on a pair of new gloves and bring the rack directly to the autoclave in the dirty cage wash area.
- Immediately autoclave the dirty cages (1 hour at 121°C/250 °F, 15psi of steam pressure). Once the autoclave cycle is completed, the cages can be emptied and the bedding disposed of in a normal fashion.

****In cases where the use of autoclave (within the animal facility) is not an option:**

- The cages (bedding) must be emptied inside the BSL-2 cabinet, directly to a double biohazard bags.
- Before closing the bags, carefully, add a small amount of water (250ml) to improve the sterilization process.

Do not close the bag completely/tightly (in order to avoid entering of steam during the sterilization process).

- Spray the dirty bag with 0.5% bleach or virusolve.
- Remove from the safety cabinet and place on a transfer rack/container.

	<ul style="list-style-type: none"> Put on a pair of new gloves and bring the rack/container, directly to the collection point of your department.
<p>8. Decontamination</p>	<p>SUSCEPTIBILITY TO DISINFECTANTS: HSV virus is easily inactivated by lipid solvent. It can be inactivated by Listerine (1:1 mixtures) in 5 min; by 2,000 ppm (2,000 ul/liter) of bleach in 10 min. HSV is also susceptible to quaternary ammonium compounds. Most herpes viruses are also susceptible to 70% ethanol and isopropanol, and 0.04% glutaraldehyde.</p> <p>PHYSICAL INACTIVATION: HSV virus is easily inactivated by exposure to PH <4, temperatures >56 °C for 30 min, pasteurization (60 °C for 10 h), and microwave heating for 4 min. HSV-2 is more heat sensitive than HSV-1.</p> <p>Susceptible to moist heat at 121 °C for 20 minutes.</p>
<p>9. Spill and Accident Procedures</p>	<ol style="list-style-type: none"> Evacuate area, remove contaminated PPE and allow agents to settle for a minimum of 30 minutes. Initiate spill response procedure. Cover the spill with absorbent material. Starting at the edges and work towards the center. Carefully pour disinfectant over the absorbed spill, again starting at the edges. Saturate the area with disinfectant. Allow sufficient contact period to inactivate the material in the spill. Non-viscous spills require 15-20 minutes: viscous spills require 30 minutes. Use paper towels to wipe up the spill, working from the edge to center. Use tongs or forceps to pick up broken plastics, glass or other sharps that could puncture gloves. Discard absorbent material in Chemical waste bags. Clean the spill area with fresh paper towels soaked in disinfectant. Thoroughly wet the spill area, and wipe with towels. Discard all cleanup materials in Chemical bag, along with any contaminated PPE (pay special attention to gloves and shoe covers). Close and secure the bag. Place bag in a second Chemical bag, secure and dispose as chemical waste. Discard contaminated PPE (with biohazard materials) in biohazard bag. Place bag in a second biohazard bag, secure and disinfect by autoclaving.

	<p><u>Exposure:</u></p> <p>Acyclovir can be used as a prophylactic drug.</p> <p>Prophylaxis with oral acyclovir is recommended to suppress genital HSV recurrences near the end of pregnancy.</p> <ol style="list-style-type: none"> 1. In case of skin contact or injection with HSV, wash the affected area with soap and water for at least 15 minutes. 2. For eye exposure, flush with water for at least 15 minutes. Consult with Employee Health Center,. Report incident to supervisor. Supervisor reports the accident/injury to the Biosafety Unit. (Eye infections associated with HSV infection can be treated either with topical trifluridine, idoxuridine, and vidarabine; or with oral acyclovir, valacyclovir, famciclovir)
10. Waste Disposal	Autoclave all waste (1 hour at 121°C/250 °F, 15psi of steam pressure).
I hereby confirm that I have read the SOP (Standard Operating Procedure) for Working with HSV in Animals, and agree to follow these procedures.	
Name:	Title:
Signature:	Date:

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