

Tel-Aviv University –Safety Unit

Standard Operating Procedure for Working with **pseudorabies virus Vectors** in Animals

<p>1. Health hazards</p>	<p>Pseudorabies, also known as Aujeszky's disease, is a viral disease caused by Suid herpesvirus 1 (SHV-1), a member of the subfamily Alphaherpesvirinae and the family Herpesviridae.</p> <p>It infects the central nervous system and other organs, such as the respiratory tract, in a variety of mammals ,except humans and the tailless apes.</p> <p>Host Range: It sporadically infects a variety of species (cattle, sheep, goats, dogs, cats, mink, foxes, raccoons and rats), causing a fatal neurological disease with rabies-like signs and severe itching. Another name for the disease in cattle is "mad itch".</p> <p>Since pseudorabies is a herpes virus; once an animal is infected, it remains infected for life and it may not demonstrate any signs of disease even though it is shedding the virus.</p> <p>The virus is spread mainly via the respiratory route and nose-to-nose contact. Transmission can also occur by contaminated drinking water, coming in contact with contaminated clothing, footwear, or equipment, especially in cool, damp weather which helps virus survival.</p> <p>There is no human health risk associated with pseudorabies.</p> <p>Zoonosis: pseudorabies is known to cause direct disease in animals .</p> <p>Infective Dose: Unknown but can be aerosol transmitted.</p>
<p>2. Housing and Biosafety consideration</p>	<p style="text-align: center;">ABSL-2</p>
<p>3. Training</p>	<p>Practical experience with animal care/maintenance, as well as general biosafety, is required.</p>
<p>4. Personal Protective Equipment (PPE)</p>	<p>Gloves ,Eyes safety goggles, Lab coat, Disposable shoe covers and Animal handling gown.</p>
<p>5. General . Precautions for Animal Use</p>	<p>The main goal is to prevent infecting the animals found at animal house.</p>
<p>6. Environmental / Ventilation Controls</p>	<p>Work should be conducted in ABSL-2 facility, over absorbent pads in a class II type A1 or A2 biological cabinet.</p>

<p>7. Animal handling practices</p>	<p>Mice are permissive host for the viral vector.</p> <ol style="list-style-type: none"> 1. Animals must be housed in filter top cages marked as biohazards (including the name of the pathogen/biohazard and date of administration). 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 pseudrabies virus . It can survive on dry inanimate surfaces (survival ranges from few hours to 8 weeks). They survive longer at lower humidity. 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: <ul style="list-style-type: none"> • place the cage containing the animals, under the biological safety cabinet and transfer the animals into a clean cage. • spray the dirty cage with 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 virusolve, and cover the rack. Put on a pair of new gloves and bring the rack directly to the autoclave. • 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. <p>**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.</p> <p>Alternately : transport the bags of cages to a HEPA filtered dumping station that draws air away from the use or fume hood.</p> <p>Mucosal protection must be worn anytime contaminated materials/equipment is handled outside a BSC.</p> <p>**Before closing the bags, carefully, add a small amount of water (250ml) to improve the sterilization process.</p> <p><i>Do not close the bag completely/tightly (in order to aloud entering of steam during the sterilization process).</i></p> <p>Spray the dirty bag with 0.5% bleach or virusolve.</p> <p>Remove from the safety cabinet and place on a transfer rack/container.</p> <p>Put on a pair of new gloves and bring the rack/container, directly to the collection point of your department.</p>
<p>8. Decontamination</p>	<p>Disinfection: 1% bleach (recommended)</p> <p>The virus is easily inactivated by lipid solvents, by 0.5% of bleach in 30 min. pseudorabies is also susceptible to quaternary ammonium compounds. Most herpes viruses are also susceptible to 30% ethanol and isopropanol, 0.12% orthophenyl phenol, and 0.04% glutaraldehyde.</p>

<p>9. Spill and Accident Procedures</p>	<ol style="list-style-type: none"> 1. Evacuate area, remove contaminated PPE and allow agents to settle for a minimum of 30 minutes. Initiate spill response procedure. 2. Cover the spill with absorbent material. Starting at the edges and work towards the center. 3. Carefully pour disinfectant over the absorbed spill, again starting at the edges. Saturate the area with disinfectant. 4. Allow sufficient contact period to inactivate the material in the spill. Non-viscous spills require 15-20 minutes; viscous spills require 30 minutes. 5. 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 6. Discard absorbent material in Chemical waste bags. 7. Clean the spill area with fresh paper towels soaked in disinfectant. Thoroughly wet the spill area, allow to disinfect for 15-20 minutes longer, and wipe with towels. 8. Discard all cleanup materials (soaked with disinfectant) in Chemical bag, and any contaminated PPE (pay special attention to gloves and shoe covers) in a biohazard bag. Close and secure the bags. 9. Place bag in a second biohazard bag, secure and disinfect by autoclaving. <p><u>Exposure:</u></p> <ol style="list-style-type: none"> 1. In case of skin contact or injection with pseudorabies, wash the affected area with soap and water for at least 15 minutes. Consult with Employee Health Center. 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.
<p>10. Waste Disposal</p>	<p>Autoclave all waste (1 hour at 121°C/250°F, 15psi of steam pressure).</p>
<p>I hereby confirm that I have read the SOP (Standard Operating Procedure) for Working with pseudorabies virus Vectors in Animals, and agree to follow these procedures.</p>	
<p>Name:</p>	<p>Title:</p>
<p>Signature:</p>	<p>Date:</p>

Dr. Esther Michael - Biological Safety Office, : 640-9966