



January 21

SOP # 009 for Working with Pseudorabies Virus

Standard Operating Procedure for Working with Pseudorabies Virus in Animals	
Health Hazards	<p>Pseudorabies, is a viral disease, also known Aujeszky's disease caused by Suid herpesvirus 1 (SuHV-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 (aerosol) and nose-to-nose contact. Transmission can also occur by contaminated drinking water, encountering contaminated clothing, footwear, or equipment, especially in cool, damp weather which helps virus survival.</p> <p>Zoonosis: pseudorabies is known to cause direct disease in animals.</p> <p>Infective Dose: Unknown but can be aerosol transmitted</p> <p>* Pseudorabies virus is NOT A RABIES VIRUS; It is a herpesvirus that predominantly infects swine, but can also infect a range of other mammals, including rodents.</p>
Designated Area	ABSL2 Facility (Animal Biosafety Level 2)
Training Requirements	<p>The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures.</p> <p>Practical experience with animal care and maintenance are required in addition to reading this SOP</p>
Personal Protective Equipment (PPE)	Gloves, safety goggles, Lab coat, disposable shoe covers and P3 respirators (masks).
General Precautions for Animal Use	It is of high importance to work carefully to prevent infecting other animals at Animal Facility
Environmental / Ventilation Controls	A. Work with animals should be conducted in ABSL-2 facility , over absorbent pads in a Class II type A2 BSC.
Special Handling Procedures	Virus injections are prepared under a biological safety cabinet (BSC), while surgeries and injections outside the BSC. (Fill the Hamilton syringe with PRV-152 virus, return to the surgery platform with the Hamilton syringe to begin the injections)



Disinfection	1% bleach (recommended); 4% glutaraldehyde
Precautions for Animal Use	<p>A. Mice are permissive host for the viral vector.</p> <p>B. NO recapping needles.</p> <p>C. Have a sharps container in close vicinity.</p> <p>D. Hands must be washed upon exiting animal room</p>
Animal Handling Practices	<p>A. Animals must be housed in filter top cages marked as biohazards), in negative pressurized IVC.</p> <p>B. Handling the cages (including bedding) will be done only by the researchers</p> <p>C. Always use a class II A2 BSC when performing work on these animals and/or when moving animals from dirty to clean cages.</p> <p>D. Injection animals with: Pseudorabies virus/vector:</p> <ol style="list-style-type: none"> 1. Injection of Pseudorabies virus/vector will be conducted within Class II A2 BSC or if no plausible use P3 respirators. 2. All needles will be disposed of in sharps container - do not recap or bend needles. 3. Injected animals considered hazardous after administration of the virus. They may shed the pseudorabies virus which can survive on dry inanimate surfaces (survival ranges from few hours to 8 weeks, survive longer at low humidity). 4. Take precautions to avoid the creation of aerosols when changing or washing cages, or cleaning the room 5. Dead animals must be placed in primary Biohazard bags which are then placed in a second Biohazard (infectious waste) and then transferred to incineration. 6. All surfaces and racks that may be contaminated will be contaminated with 0.5% sodium hypochlorite ASAP. 7. When changing cages use the following technique: <ol style="list-style-type: none"> 1. Place the cage in the BSC 2. Transfer the animals to clean cages 3. Spray the dirty cage with 0.1% sodium hypochlorite and insert the used cages in a Biohazard plastic bag. 4. Change gloves 5. Twist the ends of full bags, and seal with tape **Before closing the bags carefully, add a small amount of water (250ml) to improve the sterilization process. Do not close the bag tightly -to enable steam penetration and effective sterilization. 6. Transport the bags directly to the autoclave 7. 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 as usual.
Spill and Accident Procedures	<p>Spills must be cleaned immediately by properly protected trained personnel. Mucosal protection must be worn anytime contaminated materials/equipment is handled outside a BSC</p> <p>A. Minor Liquid Spills: should be cleaned by personnel wearing a gown, a respirator, goggles and two pairs of gloves (nitrile). Use absorbent pads to wipe liquid. The spill area should then be cleaned thoroughly with a detergent solution followed by clean water. Place waste in plastic bag and then in the chemical waste container.</p>



	<p>B. Major Spills: should be cleaned by personnel wearing a gown, a respirator, goggles and two pairs of gloves (nitrile). Personnel should be instructed to leave the laboratory and entrance should be restricted for at least 30 min.</p> <ul style="list-style-type: none"> -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 (at least 30 min) to inactivate the material in the spill -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 damage/puncture gloves. -Collect and discard all cleanup materials and contaminated PPE and place waste in a Biohazard plastic bag -Place it in a second Biohazard bag, secure and disinfect by autoclaving -The spill area should then be cleaned thoroughly with a detergent solution followed by clean water- prevent runoff into drains.. <p>A. Exposure:</p> <ul style="list-style-type: none"> • In case of skin contact or injection with pseudorabies, wash the affected area with soap and water for at least 15 minutes. For eye exposure, flush with water for at least 15 minutes. <p><u>In any case</u></p> <ul style="list-style-type: none"> • Consult a physician • Report incident to supervisor • Report the accident/injury to the Safety Unit Tel: 2146/7.
Waste Disposal	Dispose all waste as hazardous Biological material.
I hereby confirm that I have read the SOP (Standard Operating Procedure) for Working with Pseudorabies Virus in animals and agree to follow these procedures.	
<p> Name: _____ Title: _____ </p>	