Tel-Aviv University –Safety Unit

Standard Operating Procedure for Working with Vesicular Stomatitis Virus (VSV) in Animals.

1. **Health hazards**	VSV is a virus in the family Rhabdoviridae, genus Vesiculovirus. VSV is a bullet-shaped, enveloped virus, approximately 70 nm in diameter and 170 nm in length, and has a single-stranded, negative-sense RNA genome. VSV has eight main serotypes: Indiana, New Jersey, Cocal, Alagoas, Isfahan, Chandipura, Maraba, and Piry. The 11-kb genome of VSV encodes five proteins: nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and large polymerase (L). Non-exotic strains of the Vesicular stomatitis virus (VSV), including VSV-Indiana 1 serotype strains (e.g., Glasgow, Mudd- Summers, Orsay, San Juan) and VSV-New Jersey serotype strains (e.g., Ogden, Hazelhurst) are classified by the National Institutes of Health (NIH) guidelines Risk Group 2 agents, thus requiring biosafety level 2 (BSL-2) environment. **Host Range:** Humans (except for Maraba and Cocal viruses), horses, cattle, pigs, mules, sand flies, grasshoppers, and rodents. **Zoonosis:** yes, humans can contract VSV through direct contact with infected animals, or indirectly through the bite of an infected fly. **Vectors:** Sand fly (*Phlebotomus* spp.) appears to be the most important vector for VSV. Black flies (*Simuliidae*), midges (*Culicoides* spp.), mosquitoes (*Aedes* spp.) and other diptera have also been implicated. **Mode of Transmission**: Bite of an infected sand fly. By direct contact with abrasions on the skin, by contact with infected animals, or by inhaling aerosols via the nasopharyngeal route. The virus has also been transmitted via accidental autoinoculation or inhalation of aerosols in a laboratory setting. **Primary Hazards:** Exposure of skin and mucous membranes to VSV by direct contact or bite by an infected sand fly. **Susceptibility to Disinfectants:** VSV is inactivated by 1% sodium hypochlorite, 0.4% HCl 70% ethanol. **Physical Inactivation**: Inactivated by moist heat (20 minutes at 121° C) and dry heat (1 hour at 160-170°C). Inactivated at low pH (1.5), and immediately upon heating to 60°C **Survival Outside Host:** Can survive for 3 to 4 days in saliva on milking pails, barns and hay.

2. **Housing and Biosafety consideration.** **Risk Group Classification**	Chandipura and Piry viruses are classified as **Risk Group 3 human pathogens.** Indiana, Cocal, Alagoas, New Jersey, Isfahan and Maraba viruses are classified as **Risk Group 2 human pathogens.** For VSV strains except the Chandipura and Piry viruses, the work must be done under ABSL-2 containment. **Working with BSL-3 strains is prohibited at Tel-Aviv University.**
3.**Training**	Practical experience with animal care/maintenance, as well as general biosafety, is required.
4. **Personal Protective Equipment (PPE)**	Gloves (consider double-gloving), Eyes safety goggles, Lab coat, Disposable shoe covers and Animal handling gown. N-99 respirator mask covering the mouth and nose when not working in a Class II Biosafety Cabinet (BSC). Appropriate PPE should also be used for lower arms such as sleeve covers or securing gloves over the sleeves of laboratory coat.
5.**General. Precautions for Animal Use**	Tools (as, syringe, blades and safety needles where possible) should be adapted for ABSL-2. Have a sharps container in close vicinity. Animals should be restrained or anesthetized during injection.
6. **Environmental / Ventilation Controls**	Work should be conducted in ABSL-2 facility, over absorbent pads in a class II type A1 or A2 biological cabinet.
7. **Animal handling practices**	**Mice are permissive host for the viral vector.** 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 VSV for the first six days 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 1% bleach followed by water, 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 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 121ºC/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. Alternately: transport the bags of cages to a HEPA filtered dumping station that draws air away from the use (it is recommended to use a mask) or fume hood. Mucosal protection must be worn anytime contaminated materials/equipment is handled outside a BSC. **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 aloud 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. Put on a pair of new gloves and bring the rack/container, directly to the collection point of your department.
8.Decontamination	Disinfection: 1:10 bleach:water, 70 % ethanol. Decontaminate work areas with 1% bleach (2% glutaraldehyde is also candidates) for 30 minutes. Follow with water.
9. Spill and Accident Procedures	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 requite 15-20 minutes: viscous spills requite 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. <u>Exposure</u>: 1. In case of skin contact or injection with VSV, 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.

10. **Waste Disposal**	Autoclave all waste (1 hour at 121ºC/250°F, 15psi of steam pressure).

I hereby confirm that I have read the SOP (Standard Operating Procedure) for Working with Vesicular Stomatitis Virus in Animals, and agree to follow these procedures.

Name:	**Title:**
Signature:	**Date:**

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