

Tel-Aviv University –Safety Unit

Standard Operating Procedure for Working Sindbis virus (SINV) in Animals.

1. Health hazards	<p>Sindbis virus (SINV) is an envelope and nucleocapsid virus, of the genus Alphavirus, and a member of the Togaviridae family. The non-segmented genome consists of a single molecule of linear positivesense single-stranded RNA.</p> <p>SINV causes mild, febrile disease with vesicular exanthema (rash) and arthralgia. The ankle, finger, wrist, and knee joints are most affected during the acute phase. A maculopapular and, later, vesicular rash develops on body and limbs.</p> <p>Currently there are no vaccines or antiviral drugs against this virus.</p> <p>SOURCES/SPECIMENS: The virus can be isolated from infected mosquitoes, skin lesions and whole blood or serum samples of febrile patients.</p> <p>Sindbis virus can also be found in the central nervous system, blood, and liver of birds.</p> <p>MODE OF TRANSMISSION:</p> <p>SINV is transmitted via the bite of numerous ornithophilic mosquito species (Anopheles, Mansonia, Aedes, Culiseta and Culex species). Sindbis virus has also been isolated from ticks suggesting that they may also transmit the virus.</p> <p>HOST RANGE:</p> <p>Humans; a wide variety of vertebrates, wild birds (resident or migratory), and, on occasion, small mammals and amphibians</p> <p>ZONOSIS: Yes, indirectly via mosquitoes.</p> <p>VECTORS: Primarily Aedes, Culex, and Culiseta spp., and possibly ticks.</p> <p>SURVIVAL OUTSIDE HOST: Sindbis virus can infect and survive in cell culture at low temperature and low pH. SINV can also survive in various biological materials for long periods of time.</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 needlestick.</p> <p>Since the virus is spread by mosquitoes and ticks, it is forbidden to preserve any waste (that may have carried the virus) after the experiment is over. The waste must be sterilized at the end of the day.</p> <p>When the biohazard container is not in used, it should be completely covered, to prevent access of insects.</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: Sindbis virus can infect and survive in cell culture at low temperature and low PH. It can also survive in various biological materials for long periods of time.</p>
7. Animal handling practices	<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). Handling the cages (including bedding) will be done only by the researchers. 2. always Use a class II Biological Safety Cabinet (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 SINV 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: <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 0.5% bleach (or virusolve), remove from the safety cabinet and place on a transfer rack.

	<ul style="list-style-type: none"> • 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. <p>**In cases where the use of autoclave (within the animal facility) is not an option:</p> <ul style="list-style-type: none"> • the cages (bedding) must be emptied inside the BSL-2 cabinet, directly to a double biohazard bag. • Before closing the bags, carefully add a small amount of water (250ml) to improve the sterilization process. <p><i>Do not close the bag completely/tightly (in order to avoid entering of steam during the sterilization process).</i></p> <ul style="list-style-type: none"> • Spray the dirty bag with 0.5% bleach. • 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.
<p>8. Decontamination</p>	<p>SUSCEPTIBILITY TO DISINFECTANTS: Sensitive to 70% (v/v) ethanol, sodium hypochlorite (500 to 1,000 ppm free chlorine), accelerated hydrogen peroxide, and quaternary ammonium compounds.</p> <p>PHYSICAL INACTIVATION: The virus is sensitive to temperatures above 58°C. Inactivated by moist heat (15 minutes at 121° C) and dry heat (1 hour at 170° C).</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.

	<ol style="list-style-type: none"> 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, and wipe with towels. 8. 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. 9. Place bag in a second Chemical bag, secure and dispose as chemical waste. 10. Discard contaminated PPE (with biohazard materials) in biohazard bag. Place bag in a second biohazard bag, secure and disinfect by autoclaving. <p>Exposure:</p> <ol style="list-style-type: none"> 1. In case of skin contact or injection with SINV, 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.
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 SINV in Animals, and agree to follow these procedures.	
Name: Title:	
Signature: Date:	

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