Tel-Aviv University –Safety Unit

Standard Operating Procedure for Working with **Epizootic hemorrhagic disease virus (EHDV)** in Animals.	
1. Health hazards	Epizootic hemorrhagic disease virus (EHDV) belongs to the genus Orbivirus, a member of the family Reoviridae; double-stranded RNA, non enveloped viruses. Seven serotypes (1, 2 and 4-8) are currently recognized. EHDV is the causative agent of Epizootic Hemorrhagic Disease, an acute, infectious, and often fatal disease of wild ruminants. Like some other viruses, epizootic hemorrhagic disease viruses can reassort and recombine to produce new variants. Many, or perhaps all, of the serotype 6 viruses in North America are reassortants between serotype 2 and serotype 6 viruses. EHDV is closely related to bluetongue virus, a factor that can influence the development and/or selection of some diagnostic tests. Ibaraki disease, is caused by the Ibaraki strain of EHDV-2 (formerly Ibaraki virus). One outbreak of Ibaraki disease, in 1997-1998, is now attributed to a serotype 7 virus. EHDV can only be spread by an insect vector, usually biting midges of the genus *Culicoides* . **Host range**: EHDV can infect most wild and domestic ruminants. There is no evidence that epizootic hemorrhagic disease virus infects humans. **Mode of transmission/ Vectors:** Virus is transmitted by biological vectors, usually biting midges of the genus *Culicoides*, after an external extrinsic period of 10–14 days. In temperate regions infection is most common in the late summer and autumn during peak vector population, while infection occurs throughout the year in tropical regions. **Sources/Specimens**: • Blood of viraemic animals. • Infection in ruminants is not contagious – biological vectors (*Culicoides* sp.) are Required. • As the virus infects endothelium, all tissues of the body may be affected. **EHDV-TAU:** (according to prof. marcelo ehrlich statement) "the virus infects human cells with defects in their interferon antiviral response (many cancer cell types present this phenomenon). Its ability to infect normal human cells is greatly restricted (down to non-existent with dependence on cell type). The virus was made oncolytic through serial passaging on interferon insensitive LNCaP prostate cancer cells. This process resulted

	in a virus presenting multiple point and deletion mutations. In terms of mouse cells, EHDV-TAU have seen its ability to infect cells in culture of C57BL6 origin. We speculate that this is due to the absence of expression of the MX1 and MX2 antiviral genes in this inbred strain".
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	Tools (as, syringe, blades and safety needles where possible) should be adapted for BSL-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. **Survival Outside Host:** Very stable in blood and tissue specimens at 20°C, 4°C, and –70°C, but not at –20°C. Resistant to ultraviolet and gamma irradiation. Temperature: Extremely unstable at high temperatures. Inactivated by 50°C/3 hours; 60°C/15 minutes or 121°C /15 minutes. pH: Sensitive to pH <6.0 and >8.0 Chemicals/Disinfectants: Non-enveloped virus and thus relatively resistant to lipid solvents like ether and chloroform. Readily inactivated by ß-propiolactone, 2% w/v glutaraldehyde, acids, alkalis (2% w/v sodium hydroxide), 2-3% w/v sodium hypochlorite, iodophores and phenolic compounds.
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 EHDV after treatment; take precautions to avoid the creation of aerosols when changing or washing cages, or cleaning the room. EHDV is a non-enveloped virus therefore has a relatively high survival in the environment; therefore the cages and the bedding will be considered as biohazards, for the whole time. **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 ASAP (or virusolve). **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℃/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 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	EHDV is relatively resistant to lipid solvents like ethanol, ether and chloroform. Readily inactivated by ß-propiolactone, 2% w/v glutaraldehyde, acids, alkalis (2% w/v sodium hydroxide), **2-3% w/v sodium hypochlorite** (freshly made), iodophores and phenolic compounds. **Physical inactivation:** extremely unstable at high temperatures. Inactivated by 50°C/3 hours; 60°C/15 minutes or 121°C /15 minutes.
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, 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. **<u>Exposure</u>:** 1. In case of skin contact with EHDV, wash the affected area with soap and water for at least 15 minutes.

	2. In case of injection with EHDV: Rinse thoroughly for 15 minutes, while pressing the area of injury to draw blood out, disinfect with of iodine solution, notify the safety unit and get medical care. 3. 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 EHD virus in Animals, and agree to follow these procedures.	
Name:	Title:
Signature:	Date:

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