

Tel-Aviv University –Safety Unit

Standard Operating Procedure for Working with Vaccinia Virus (VACV) in Animals.

1. Health hazards

The vaccinia virus (VACV) is an enveloped, linear, double stranded DNA virus that is a member of the Poxviridae family.

Vaccinia Virus, normally has no serious health effects in humans, although it can cause disease of the skin when used to inoculate against the smallpox virus.

The virus can be a pathogen in individuals with immunological and dermatological abnormalities, and on occasion can cause serious problems in normal hosts.

Host Range: Humans and several mammals, including humans, rabbits and cows.

Zoonosis: yes.

Mode of Transmission: Vaccinia virus transmission between humans occurs through direct contact, Ingestion, parenteral inoculation, droplet or aerosol exposure to mucous membranes (respiratory secretions), and exposure to broken skin.

Infected tissues or animals containing the virus are also a way for laboratory - acquired infections.

Susceptibility to Disinfectants: Susceptible to 10% bleach, 70% ethanol.

The virus is however resistant to solvent/detergent combinations (TNBP/Triton X-100 and TNBP/ Tween 80).

Physical Inactivation: The virus is inactivated by dry heat at 95 °C for 2 hours and moist heat (autoclave) 120°C for 15 min.

The virus in its aerosol form is also sensitive to UV light (254 nm).

Survival Outside Host: The dried virus can survive up to 39 weeks at 6.7% moisture and 4°C. Lyophilized VACV remains potent for 18 months at 4-6°C.

Immunization: Smallpox vaccination is recommended for laboratory personnel working with the vaccinia virus (the smallpox vaccine and tissues, materials or animals that may be infected) or other orthopoxviruses because the virus can be spread to non-vaccinated individuals.

Vaccination is recommended every 10 years.

The time it takes to become immune is usually 7-14 days after vaccination.

Vaccines are contraindicated in immunocompromised people, those with particular skin (eczema) and heart conditions, and pregnant women.

Containment Requirements: BSL-2 facilities, equipment, and operational practices are recommended when working with the vaccine.

All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).

	<p>Genetically engineered recombinant vaccinia viruses pose an additional potential risk to laboratory personnel, through direct contact or contact with clinical materials from infected volunteers or animals.</p> <p>Personal Protective Equipment (PPE): Lab coat and gloves (particularly when there is direct physical contact with contaminated materials or animals). Eye protection must be used where there is a known or potential risk of exposure to splashes.</p> <p>The use of needles, syringes, and other sharp objects should be strictly limited. Additional precautions should be considered with work involving animals or large-scale activities.</p>
2. Housing and Biosafety consideration	The work must be done under ABSL-2 containment
3. Training	Practical experience with animal care/maintenance, as well as general biosafety, is required.
4. Personal Protective Equipment (PPE)	<p>Gloves (consider double-gloving), Eyes safety goggles, Lab coat, Disposable shoe covers and Animal handling gown.</p> <p>N-99 respirator mask covering the mouth and nose when not working in a Class II Biosafety Cabinet (BSC).</p> <p>Appropriate PPE should also be used for lower arms such as sleeve covers or securing gloves over the sleeves of laboratory coat.</p>
5. General. Precautions for Animal Use	<p>Tools (as, syringe, blades and safety needles where possible) should be adapted for BSL-2. Have a sharps container in close vicinity.</p> <p>Animals should be restrained or anesthetized during injection.</p>
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	<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 VACV for the first three 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 or virusolve ASAP. 6. When changing cages, use a standard microisolator technique:

	<p>place the cage containing the animals, under the biological safety cabinet and transfer the animals into a clean cage.</p> <p>spray the dirty cage with virusolve, remove from the safety cabinet and place on a transfer rack.</p> <p>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.</p> <p>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> <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 (it is recommended to use a mask) 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 avoid 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: 10% bleach (recommended)</p> <p>Decontaminate work areas with 1% bleach (2% glutaraldehyde is also candidates) for 30 minutes. Follow with water.</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 VACV, 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 121°C/250 °F, 15psi of steam pressure).

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

Name:	Title:
Signature:	Date:

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