

Tel-Aviv University –Safety Unit

Standard Operating Procedure for Working with AAV(Adeno-associated virus) Vectors in Animals

This document applies to cases, which the safety unit defines as **BSL-2**

1. Health hazards	<p>Adeno-associated virus (AAV) is a small virus which infects humans and some other primate species.</p> <p>AAV belongs to the genus Dependoparvovirus, which in turn belongs to the family Parvoviridae. The virus is a small (20 nm) replication-defective, nonenveloped virus. AAV has a genome consisting of single-stranded DNA and an icosahedral protein capsid.</p> <p>AAV is not known to cause disease and consequently the virus causes a very mild immune response.</p> <p>Wild type AAV virus is dependent for replication on the presence of adenovirus or herpesvirus and will, in the absence of helper virus, stably integrate into the host cell genome, at a specific site on the human chromosome 19 and remain latent. Potentially at a later time when a helper virus is present, AAV can be reactivated and produce infection.</p> <p>Therefore, AAV may not be as safe as previously thought.</p> <p>Host Range: Humans and animals</p> <p>Zoonosis: AAV is not known to cause direct disease in humans .</p> <p>Clinical Manifestation: None</p> <p>Infective Dose: Unknown but can be aerosol transmitted</p> <p>Cell types able to be infected/transduced: A wide range of host of human and non-human cell lines cells (dividing and non-dividing).</p> <p><u>Host-vector systems</u></p> <p>Vector information: The long terminal repeats flank the transgene, and the AAV Rep and Cap and helper virus genes are provided in trans. In the absence of helper virus, recombinant AAV will stably integrate into the host cell genome so expression of the transgene is long term and stable.</p> <p>Helper virus: Adenovirus, herpesvirus, vaccinia virus or CMV, for replication</p> <p>Deletion of specific genes: Allows AAV to insert specifically in other chromosomal sites.</p>
2. Housing and Biosafety consideration	<p>The work must be done under ABSL-2 containment in the following cases:</p> <ol style="list-style-type: none"> 1. When a known helper virus is present or the host animal may potentially contain virus that could act as a helper (e.g. mice replete with retroviruses). 2. For recombinant AAV: Because residual helper virus may not be completely inactivated during AAV purification, helper virus may be present . 3. When AAV vector expressing highly biologically active molecules such as

	<p>oncogenes (including siRNA to a tumor suppressor) allergens or cytokines.</p> <p>The NIH classifies the following AAV vectors as BSL-1:</p> <ol style="list-style-type: none"> a. adeno-associated virus (AAV) types 1 through 4 b. recombinant AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product (oncogene) or a toxin molecule c. also are produced in the absence of a helper virus.
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> <p><i>Personnel should not work with AAV, if skin is cut or scratched.</i></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 AAV 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: <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 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 121°C/250° F, 15psi of steam pressure). Once the autoclave cycle is completed, the cages can be emptied and the

	<p>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>
8. Decontamination	<p>Disinfection: 10% bleach (recommended)</p> <p>Decontaminate work areas with 1% bleach or virusolve (2% glutaraldehyde is also candidates) for 30 minutes. Follow with water.</p>
9. Spill and Accident Procedures	<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 AAV, 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	<p>Autoclave all waste (1 hour at 121°C/250 °F, 15psi of steam pressure).</p>

<p>Comments regarding to research on bats.</p>	<p><u>Comments regarding to research on bats.</u></p> <ol style="list-style-type: none"> 1. Any manipulation on the bats, including anesthetization with isoflurane, injection the rAAV and autopsy, will be done in a dedicated room in the researcher's lab. 2. The cages where the bats will be stored should be marked as biohazards (including the name of the pathogen/biohazard and date of administration). 3. The cage's floor, where the injected bats will be stored should be covered with absorbent pads (with nylon on the bottom). 4. Infected animals may shed rAAV for the first three days after treatment; take precautions to avoid the creation of aerosols when entering the cage. Do not transfer the infected bats prior to these 3 days. 5. Use the following PPE following entrance to the cages: Gloves (consider double-gloving), Eyes safety goggles, Lab coat, Disposable shoe covers, Animal handling gown and N-99 respirator mask (covering the mouth and nose). 6. At the end of the experiment or 3 days after injecting the rAAV, the bedding (on the absorbent pads) need to be picked up into biohazard bag and transferred to an autoclave. <p>One should be reminded that rAAV particles are stable in a wide pH range (3 to 9) and can remain infectious for at least a month at room temperature following simple desiccation.</p> <p style="text-align: center;">At the end of the experiment:</p> <p style="text-align: center;">Use a Vaporized Hydrogen Peroxide for the cage.</p> <p style="text-align: center;">Use a 10% fresh bleach solution, for the cage floor, followed by an alcohol wipe to lessen the corrosive nature of the germicide.</p>
<p>I hereby confirm that I have read the SOP (Standard Operating Procedure) for Working with AAV in Animals, and agree to follow these procedures.</p>	
<p>Name:</p>	<p>Title:</p>
<p>Signature:</p>	<p>Date:</p>

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