Tel-Aviv University –Safety Unit

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

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

1. Health hazards	Adeno-associated virus (AAV) is a small virus which infects humans and some other primate species. 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. AVV has a genome consisting of single-stranded DNA and an icosahedral protein capsid. AAV is not known to cause disease and consequently the virus causes a very mild immune response. Wild type AAV virus is dependent for replication on the presence of adenovirus or herpes virus 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. Therefore, AAV may not be as safe as previously thought. **Host Range:** Humans and animals . **Clinical Manifestation:** None **Infective Dose:** Unknown but can be aerosol transmitted **Infected/transduced cells :** A wide range of human and non-human cell lines cells (dividing and non-dividing). <u>Host-vector systems</u> **Vector information:** The AAV vector particles are generated by transfecting packaging cells with a plasmid (AAV cis-plasmid) containing a cloned recombinant AAV genome composed of the transgene flanked by the AAV ITRs (inverted terminal repeats), and a separate construct expressing in trans the vital rep and cap genes. The adenovirus genes, such as E1A, E1B, E2A, E4ORF6 and VA RNA, must be provided by either adenovirus infection or plasmid. Given that HEK293 cells, commonly used vector producing cells, already contain the E1A/E1B gene, the helper genes that need to be provided by the plasmid are E2A, E4ORF6 and VA RNA. The final viral vector particle will not contain any of the genes provided by the helper plasmids, but still remains infective. Because AAV vectors are devoid of rep coding sequences, the property of site-specific integration is not remained. Instead, persistent expression of vector sequences may occur from extra chromosomal (episomal) sequences and, in lower frequency, from randomly integrated sequences. Therefore, the AAV vectors have an oncogenic/mutagenic potential.

	Helper virus: Adenovirus, herpes virus, vaccinia virus or CMV, for replication **Deletion of specific genes**: Allows AAV to insert specifically in other chromosomal sites.
2. **Biosafety consideration**	The work must be done under ABSL-2 containment in the following cases: 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 oncogenes (including siRNA to a tumor suppressor) allergens ,cytokines or toxines. The NIH classifies the following AAV vectors as BSL-1: 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	General biosafety training, is required.
4. Personal Protective Equipment (PPE)	Gloves (consider double-gloving), eyes safety goggles and lab coat. 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. ***Personnel should not work with AAV, if skin is cut or scratched.***
5.General . Precautions	Tools (as, syringe, blades and safety needles where possible) should be adapted for BSL-2. Have a sharps container in close vicinity.
6. Environmental / Ventilation Controls	Work should be conducted in BSL-2 facility, over absorbent pads in a class II type A1 or A2 biological cabinet.
7. Exposure risks	Transmission of AAV can occur through inhalation of aerosolized droplets, mucous membrane contact, ingestion and accidental injection. When handling AAV-containing cultures outside of containment equipment, a respirator (N99 mask) should be worn. Combination of googles and respirator provided adequate protection (mucosal and respiratory).
8.Decontamination	Disinfection: 10% bleach (recommended) followed by an alcohol wipe to lessen the corrosive nature of the bleach. AAV particles are stable in a wide pH rang (3-9), and can resist heating at 56^0C for 1 hour. Due to the high stability of the capsid, AAV can remain infectious for at least a month at room temperature following simple desiccation or lyophilization.

	AAV, as well as other non-enveloped viruses, is quite resistant to alcohol disinfectants. Decontaminate work areas with 0.5% bleach or virusolve ,2% glutaraldehyde or 0.25% sodium dodecyl sulfate are 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/ container, 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 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	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 AAV(Adeno-associated virus) Vectors, and agree to follow these procedures.	
Name:	Title:
Signature:	Date:

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