FACT SHEET

Reed College
Environmental Health & Safety

Adeno-Associated Viral Vectors

Adeno-associated viruses (AAV) are non-enveloped icosahedral viruses with a single stranded DNA genome used in creating viral vectors for gene transfer. Investigators must have an approved Institutional Biosafety Committee (IBC) protocol for work with recombinant AAV vectors. These guidelines may be used as part of risk assessment when planning experiments with these vectors and preparing applications to the IBC.

Pathogenesis	When wild type AAV infects human cells in the absence of a helper virus, its gene expression program is shut down and the virus remains latent by stably integrating into a specific site on chromosome 19. Recombinant AAV vectors do not contain Rep coding sequences and so lose the property of site-specific integration, raising the concern of insertional mutagenesis. Upon subsequent infection of the cell with a helper virus (CMV, adenovirus, herpesvirus, vaccinia), the AAV genome is rescued from latency, resulting in AAV DNA replication and the formation of new viral particles.
Exposure Potential	Most adults (85-90% in the US) are seropositive for AAV and about 30% have neutralizing antibodies. • Exposure to AAV in the lab may occur through skin or mucous membrane contact, accidental injection, inhalation and ingestion. • AAV may be transmitted through direct contact with an infected individual or through indirect contact with the contaminated environment • A concern for vertical transmission from mother to fetus also exists.
Personal Protective Equipment (PPE)	Lab coat, gloves, eye protection. If a helper virus is used, do aerosolization (centrifuge) and aliquots in a biosafety cabinet.
Health Hazards	Though AAV is not known to cause disease in humans, precautions must be taken due to the possibility of insertional mutagenesis. • There is evidence of AAV infection in the human embryo and an association of AAV with male infertility. • A significant correlation was found between the presence of AAV in amniotic fluids and premature amniorrhexis and labor.

	Potentially at a later time when a helper virus is present, inactive AAV can be reactivated and produce infection. • In the presence of helper virus, AAV can replicate and generate up to $1x10^6$ copies per cell, thus killing them. In addition, helper viruses used to trigger AAV replication may cause disease.
Biosafety Considerations	AAV are considered Risk Group 1 agents. Most work with AAV vectors can be conducted at BSL-1 or BSL-2 containment level, depending on a risk assessment. Work must be done under BSL-2 containment in the following cases: • When a helper virus is present or a host animal may contain a helper virus • For recombinant AAV (residual helper virus may not be completely inactivated during AAV purification) • When an AAV vector expresses highly biologically active molecules (ie. Oncogenes, siRNA to a tumor suppressor, allergens, cytokines, or toxins)
Animals	ABSL-2 will generally be required if a helper virus is used or if the host animal could house a helper virus. • Animals must be injected in a Biological Safety Cabinet. • Special handling of bedding and cages is required for 48 hours following injection. Animals can be transferred to ABSL-1 conditions 72 hours following infection. • They should be transferred to a clean cage and the ABSL-2 cage remain in the ABSL-2 quarantine space for appropriate waste disposal and cleaning. • Bedding must be disposed of as biohazardous waste.
Decontamination	AAV particles are stable in a wide pH range (3 to 9) and can resist heating at 56°C for 1 hour. • Due to the high stability, AAV can remain infectious for at least a month at room temperature after desiccation or lyophilization. • Since AAV is a non-enveloped virus, it is very resistant to alcohol-based disinfectants. A freshly prepared 10% bleach solution should be used as a disinfectant instead. NOTE: Effective disinfectants require a minimum of 20 minutes contact time.