



ADENO-ASSOCIATED VIRUS AND ADENO-ASSOCIATED VIRAL VECTORS

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Reference: <http://www.uiowa.edu/~hpo/>

Adeno-associated viruses (AAV) belong to the family Parvoviridae. They are non-enveloped, single-stranded DNA viruses that can only replicate in the presence of a helper virus, adenovirus (Ad), herpes virus, or vaccinia. Wild-type AAV may integrate into the host-cell genome (preferentially into human chromosome 19) and remain latent until a helper virus supplies the necessary genes for replication.

AAV-vector characteristics include:

- A limited cloning capacity (~4.5kb).
- Ability to be produced in high titers.
- Ability to infect a broad range of cells.
- Long-term (stable) expression from randomly integrated sequences or episomal sequences.
- Replication in the presence of wild-type AAV and of a helper virus.

Potential Health Hazards

AAV is not associated with any human disease; however, 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 DNA in amnion fluids and premature amniorrhexis (rupture of the amnion) and premature labor.

Recombinant AAV vectors lose site specific integration into chromosome 19, thereby raising the theoretical concern of insertional mutagenesis.

Modes of Transmission

AAV may be transmitted through direct contact with an infected individual or through indirect contact with the contaminated environment. Transmission routes include respiratory, gastrointestinal and possibly sexual transmission. A concern for vertical transmission from mother to fetus also exists.

Most adults (85-90% in the US) are seropositive for AAV and about 30% have neutralizing antibodies.

Laboratory Acquired Infections

There is a theoretical risk of infection from exposure to laboratory cultures of wild-type adeno-associated virus or recombinant viruses. Transmission of AAV can occur through ingestion, inhalation of aerosolized droplets, mucous membrane contact and accidental injection (for example, as the result of a needlestick).

Host Range

Recombinant AAV vectors can infect a wide range of cell types from a variety of mammalian cells.

Survival

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 of the capsid, AAV can remain infectious for at least a month at room temperature following simple desiccation or lyophilization.

Laboratory Practices

Biosafety level 2 practices and facilities must be used for activities involving adeno-associated viruses/viral vectors, as determined by the Institutional Biosafety Committee (IBC, rDNA Committee).

- Biohazard signs and labels must be displayed in areas and on equipment where AAV is used and stored. This includes, but is not limited to, laboratory entrance doors, biological safety cabinets, incubators, refrigerators, and freezers.
- Use a biological safety cabinet (BSC) (a.k.a. tissue culture hood) for manipulations that can generate aerosols, such as pipetting, harvesting, infecting cells, filling tubes/containers, and opening sealed centrifuge canisters. If a procedure cannot be done in a BSC and only on an open bench, use a plastic shield to prevent exposure through inhalation or splashing.
- Use aerosol containment devices when centrifuging. These include sealed canisters that fit in the centrifuge bucket, covers for the centrifuge bucket, heat sealed tubes, or sealed centrifuge rotors. Rotors should be removed and opened inside a BSC. Centrifuge tubes should be filled and opened in a BSC.
- Vacuum lines must be protected with liquid disinfectant traps and/or micron filters.

Personal Protective Equipment

Personal protective equipment (PPE) includes, but is not limited to:

- Disposable gloves (nitrile, latex, etc.).
- Lab coat when working in the area. Remove when leaving the laboratory.
- Goggles for splash protection.

Precautions When Using Animals

Animal use requests are made to the Institutional Animal Care and Use Committee (IACUC) by submitting an Animal Care and Use Form (ACURF) found at <http://research.uiowa.edu/animal/downloads/acurf.doc>.

When animals are infected with adeno-associated virus/vectors, the Animal Biosafety Level of the project will be generally assigned to ABSL-1. The Animal Biosafety Level protocol is sent to the principal investigator (PI).

Animal cages must be labeled with a biohazard sign.

Recombinant AAV Research

Protocols involving recombinant AAV vectors must be approved by the Institutional Biosafety Committee (IBC). Complete an “**rDNA Registration Document**” and submit it to Biosafety, HPO-100 HPO. The form is available at <http://www.uiowa.edu/~hpo/biosafety/rdnaregform.doc>.

Employee Exposure

Due to the possibility of adenovirus contamination, recommended treatment is the same for exposure to adenovirus.

Eye Exposure – Rinse eyes in an eyewash for at least 15 minutes.

Skin Exposure – Rinse skin with soap and water.

Accidental Needlestick Injury – Scrub contaminated skin with soap and water.

Report Incidents and Seek Treatment – Report actual or suspected exposure incidents to your supervisor immediately. Seek treatment at the Worker’s Health Clinic, if necessary. It is located on the first floor of Boyd Tower – General Hospital. The clinic’s phone number is 353-8653. If the incident occurs after 4:30pm, during the weekend, or on a holiday, proceed to UIHC’s Emergency Treatment Center (ETC). The phone number is 356-2233.

- **Immunizations and Prophylaxis** – none available.
- **Incubation Period** – no symptoms apparent.

Spill and Disposal Procedures

For spills **outside** a biological safety cabinet, leave the area while holding your breath. Once outside the area, wash hands and face with soap and water. Do not allow anyone inside the area or room where the spill occurred. Allow 30 minutes for the aerosols to settle. Enter the room wearing required protective clothing, gently cover the spill with paper towels, and apply disinfectant starting at the perimeter and working towards the center. Allow the disinfectant to remain on the spill for at least 20 minutes before initiating spill clean up. After initial clean up, disinfect the area a second time.

For spills **inside** a biological safety cabinet, cover the spill with paper towels or wipes. Gently pour disinfectant over the spill area. Let the disinfectant soak for 20 minutes before cleaning up the spill. After initial clean up, disinfect the area a second time.

Contaminated materials must be disposed of as biohazardous waste.

Decontaminate adjacent surfaces with a bleach solution.

Disinfectants

Effective disinfectants require a minimum of 20 minutes contact time. Use one of the following:

- Sodium hypochlorite (1-10% dilution of fresh bleach).
- Alkaline solutions at pH >9.
- 5% phenol.

Note: Alcohol is not an effective disinfectant against AAV species.

Decontamination

Autoclave cultures for 30 minutes at 121°C or 250°F (15lbs per square inch of steam pressure).

Disinfect work surfaces using an effective germicide (see above). This may be followed by an alcohol wipe to lessen the corrosive nature of the germicide.

Transport Requirements

Materials must be appropriately contained and labeled for transport within the University. Shipping infectious substances, diagnostic specimens, and/or shipping with dry ice off-campus require training and certification. See HPO's fact sheet "Transporting and Shipping Infectious Substances" found at <http://www.uiowa.edu/~hpo/biosafety/transportingguideSIS.pdf> for additional information.

If you have questions

Contact HPO's Biological Safety Section at 335-8501.

Information and References

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