UNIVERSITY OF SOUTH ALABAMA

Guidelines for Research Involving Viral Vectors: Adeno-associated Virus (AAV)

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 56oC 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 dessication or lyophilization.

Laboratory Practices

Biosafety Level 2 practices and facilities must be used for activities involving adenoviruses/viral vectors.

- Biohazard signs and labels must be displayed in areas and on equipment where adenoviruses are 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 a micron filter.

Personal Protective Equipment

Personnel protective equipment (PPE) includes, but is not limited to-

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

Precautions When Using Animals

- Inoculation of BSL-2 biohazardous materials (including, but not limited to: viral vectors and human tumor cell lines) must be performed within a Class II BSC*.
- Tissue harvest (including blood collection) must be performed in the necropsy suite or in a Class II BSC.
- ABSL-2 signage must be placed on the animal room door when BSL-2 agents are in use. See Department of Comparative Medicine for your room assignment and signage.
- Depending on hazard assessment as performed by the IBC, IACUC, and Attending Veterinarian, cages may be considered biohazardous. Please meet with Comparative Medicine prior to initiation of the animal experiments to discuss handling of soiled cages and other waste materials.
- All cages containing animals inoculated with biohazardous agents must be marked with:
    - The agent
    - The PI
    - The date of administration
    - Any special handling requirements of soiled bedding/cages.
- ABSL-2 carcasses are considered biohazardous and are incinerated.

*Deviation from using a Class II BSC must be approved by the IBC and/or IACUC Committee

Animal use requests are made to the Institutional Animal Care and Use Committee (IACUC).

A complete copy of USA's Animal Biosafety (ABSL-2) Guidelines can be found at: https://southalabama.edu/departments/research/compliance/animalcare/animal.biosafety.guidelines.pdf

Recombinant AAV Research

Protocols involving recombinant AAV vectors must be approved by the Institutional Biosafety Committee (IBC)

Employee Exposure

Eye exposure - Rinse eyes with eyewash for at least 15 minutes.
Skin exposure - Cleanse the affected skin area immediately with surgical disinfectant soap, diluted Clorox (0.05%) or other approved disinfectant.
Report Incidents and Seek Treatment - Report actual or suspected exposure incidents to your supervisor immediately. An online incident report must be completed within 72 hours of the incident. This form can be found at: https://jagasp2.southalabama.edu/incident/logon.aspx
If possible, identify and secure the offending sample to contain its biohazardous content and to allow for testing if necessary.

Spills and Disposal Procedures

• If the spill area is large or in a common use area, mark the area so that others may avoid it.
• Using materials from you spill kit:
    o Don the appropriate PPE
    o Cover the spill with absorbent material
    o Pour disinfectant over the entire area and allow to stand for 30 minutes.
• Contact the PI and assess the magnitude of the spill and formulate further plans of action.
• Safely pick up any broken glass with tongs or sweep in to a dust pan.
• Place spill material in to an autoclave bag.
• Make sure that the area is cleaned and disinfected thoroughly.
• Soak contaminated clothes and shoes in a tray with approved disinfectant.
• Report all spills containing biohazardous or recombinant material to the Office of Research Compliance and Assurance at 251-460-6863.

Disinfectants

Disinfectants should be allowed a minimum of 20-30 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.

Decontamination

Autoclave cultures for 30 minutes at 121oC or 250oF (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 Shipping and Packaging Biological Materials posted on the USA Biosafety training website for additional information.

Information and References
University of Iowa Environmental Health and Safety https://ehs.research.uiowa.edu/

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