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Rutgers Environmental Health and Safety

Adeno-Associated Viral Vectors

Background

Adeno-associated viruses (AAV) are non-enveloped icosahedral viruses composed of a linear single-stranded DNA genome. These viruses have been used as viral vectors due to their efficient gene transfer, transient or stable transgene expression, and lack of induction of strong immune responses. However, production of AAV requires a helper plasmid, usually in the form of a helper Adenovirus. A primary safety concern is the presence (contamination) of helper virus when growing these vectors. Also, if no helper virus is present, AAV can insert DNA stably into the host genome, which is usually at a specific site on chromosome 19. The insert remains latent until activated by infection of a helper virus, causing expression of the viral DNA from the host cell chromosome and creation of replicating viral progeny. In terms of a safety profile, while this insertional mutagenesis in not optimal, the site specificity is desired as it decreases risk of being oncogenic. The final vector produced from adenoviral plasmids and helper virus does not contain any genes provided by the helper virus, but still remains infective.

AAV is usually classified as a Risk Group 1 (RG1) agent, but the appropriate biosafety level determination will depend on the nature of the gene that the vector carries. AAV constructs that do not encode an oncogenic or toxic transgene can be handled at BSL-1. If helper virus is used during the production of the AAV, BSL2 containment is required.

Symptoms of Exposure

There is no known disease linked to AAV, but there is data indicating infection of the human embryo and an association with male infertility.

Modes of Transmission

AAV may be transmitted by aerosol, droplet exposure to the mucous membrane, ingestion and injection (i.e. needle stick).

Host Range

Recombinant AAV vectors infect a wide range of mammalian cells.

Environmental Stability

AAV particles are stable in a wide pH range (3 to 9) and can resist heating at 56 ºC for 1 hour. Due to the stability of the protein capsid, these viruses are stable for up to a month at room temperature. Alcohol-based disinfectants are NOT effective. A broad spectrum disinfectant, such as sodium hypochlorite (i.e. 1:10 bleach solution) must be used to inactivate AAVs.

Approvals

Experiments using AAV require IBC approvals before initiation of experiments.

Test Methods for Replication Competent Virus

**If vectors are being obtained from a commercial supplier, please check the manufacturer's information as to the quality control concerning replication competent viruses. This information should be supplied with the IBC application.

AAV - no helper: Not required

AAV with adenovirus helper: Every viral preparation MUST be tested for the presence of replication competent adenovirus prior to *in vitro* or *in vivo* use. Viral preparations can be heat inactivated for 15 minutes at 56 C and tested for the presence of replication competent adenovirus by plaque assay or cytopathic effect (*Hehir*, 1996).

**Each inoculum must be proven to be free of recombinant virus before use in animals.

Laboratory Practices

Generally, AAV is classified as a **Biosafety Level 1** (BSL-1) organism, **unless a helper virus is used**, then it would be classified as Biosafety Level 2 (**BSL-2)**. AAV vectors may be regulated at varying biosafety levels, depending on the nature of the inserted genes and its replication competence as well as the presence of a helper virus. Animal housing is BSL1, unless there is a helper virus which could be shed from the animals.

1. No work with AAV is permitted on the open bench unless approved by the IBC.

2. A primary containment device (e.g., biological safety cabinet, laboratory "fume" hood, or animal transfer station) that has been inspected within the last 12 months must be used for all manipulations including (but not limited to):
   - Pipetting
   - Harvesting infected cells for RNA
   - Infection of cell culture
   - Infection of animals (REHS will perform risk assessment for certain activities such as stereotaxic injections).

3. Centrifugation must be done in closed containers and using **sealed rotors or safety cups**. Safety cups are to be opened inside the biosafety cabinet.

4. All vacuum lines must be fitted with a HEPA filter (an example is the "Vacushield™" inline hydrophobic filter, Product #4402 from Gelman Science , Millex FH vacuum line protector Millipore (Fisher) cat #SLFH05010, or "HEPA-VENT™" inline hydrophobic filter, Catalog # 6723-5000 from Whatman).

5. All laboratory staff working with or supervising work with AAV must be made aware of the hazards associated with the work, required safety practices and procedures, and proper handling of the agent, as well as be current on required laboratory safety and biosafety training requirements.

6. Animal carcasses must be placed in autoclave bags and be designated for infectious waste disposal.

7. Signs and labels must be placed to indicate each area where AAV is used or stored (including biosafety cabinets, incubators, refrigerators, laboratory entrance doors, etc.). The signs should include the name of the agent, emergency contact information, and a biohazard sticker.
8. Reminder: All work and manipulations of AAV must be conducted in a primary containment device (e.g., biological safety cabinet, laboratory "fume" hood, or animal transfer station) inspected in the last 12 months. If there are extenuating circumstances, please contact REHS (at the numbers listed at the end of this SOP) as additional precautions may be required.

Personal Protective Equipment

1. Disposable gloves.

2. Disposable gown or equivalent when introducing vector into animals or performing necropsies. Lab coats or disposable gowns are adequate for tissue culture manipulations.

3. Eye protection.

Instructions in the Event of Employee Exposure

- **EXPOSURE FROM SPLASH OR AEROSOLS – INHALATION**
  Report the incident to your supervisor and refer to the Rutgers Emergency Action Plan for further instructions. The supervisor should submit an incident report through https://MyREHS.rutgers.edu to document the event.

- **EXPOSURE FROM SPLASH OR AEROSOLS – EYE CONTACT, SKIN AND/OR MUCOUS MEMBRANE**
  Rinse a minimum of 15 minutes in eye wash or flush area with water, report the incident to your supervisor and refer to the Rutgers Emergency Action Plan for further instructions. The supervisor should submit an incident report through https://MyREHS.rutgers.edu to document the event.

- **NEEDLESTICK AND/OR SHARPS EXPOSURE**
  Contaminated skin should be thoroughly scrubbed for several minutes with soap or a 10% povidone solution (Betadine) and copious amounts of water. Report the incident to your supervisor and REHS immediately after scrub. Seek medical attention at Campus Employee Health Services/Occupational Medicine Services. Refer to the Rutgers Emergency Action Plan for further instructions. The supervisor should submit an incident report through https://MyREHS.rutgers.edu to document the event.

- **EMERGENT EXPOSURES**
  For situations in which exposure to AAV occurred and medical treatment is an emergency, personnel should report to the Emergency Room, and ensure their supervisor completes an incident report through https://MyREHS.rutgers.edu to document the event.

Decontamination

The most effective disinfectant against AAV is a 1:10 Sodium hypochlorite (bleach) solution that is made fresh daily.

- To make this solution, dilute 1 part bleach to 10 parts tap water.
- Ensure a 15 minute contact time.
- Use this disinfectant for treatment of reusable equipment, surfaces, and liquid waste (final volume 1:10 bleach).

Disinfectant alternatives include 2% glutaraldehyde, and 0.25% sodium dodecyl sulfate (SDS).

Autoclaving for 1 hour at 121°C or 250°F (15 lbs psi of steam pressure).

- Use this disinfection method for reusable equipment, liquid waste or solid waste.
- ***NOTE:*** If equipment unavailable, follow guidelines provided by IBC.

Animal Practices

1. When animals are infected with AAV vectors, an Animal Biosafety Level - 1 (ABSL-1) area must be approved and used for the procedure. Concurrent approvals are needed from the Institutional Biosafety Committee (IBC) and the Institutional Animal Care and Use Committee (IACUC). At the discretion of the IBC, a higher BSL may be requested, depending on the presence of helper virus and the gene insert.

2. All bedding, waste and animals shall be treated as biohazardous. Cage changing and husbandry must be performed according to the hazard sign provided by REHS. All waste must be decontaminated by autoclaving or chemical disinfection prior to disposal.

3. Animal carcasses must be placed in autoclave bags and be designated for infectious waste disposal.

4. All necropsies must be performed in a designated room using animal BSL-2 practices and procedures.

5. The following information must be posted on the door of the animal room. REHS will provide a sign template to the animal facility staff for this purpose.
    - A description of special housing required to ensure safety of animal facility personnel, such as ventilated cabinets or hoods.
    - A label on the animal cage indicating the hazardous materials to be administered to live animals. (i.e., AAV w/ or w/o helper virus)
    - The name of individual(s) responsible for handling the materials**.** (i.e., Drs. X, Y and Z and Technicians A and B as per protocol #00000)
    - A description of how to handle animals, carcasses, and contaminated cages and bedding

References

CDC-BMBL, 5th ed., www.cdc.gov/od/ohs/biosfty/bmbl5/BMBL_5th_Edition.pdf

Environmental Health and Safety. The University of Iowa, " Adeno-Associated Virus and Adeno-Associated Viral Vectors" https://research.uiowa.edu/ehs/files/documents/biosafety/AAV.pdf

Hazardous and Radioactive Waste Disposal Standard Operating Procedure, Comparative Medicine Resources http://njms.umdnj.edu/research/cmr/sop.cfm

Hehir, KM, Armentano, D, Cardoaz, LM, et al. 1996. “Molecular characterization of replication-competent variants of adenovirus vectors and genome modifications to prevent their occurrence”. J. Virol. 70:8459-8467.

Stanford University, “Working with Viral Vectors,” http://www.stanford.edu/dept/EHS/prod/researchlab/bio/docs/Working_with_Viral_Vectors.pdf

Young, L.S., Searle, P.F., Onion, D., and V. Mautner. 2006. “Viral gene therapy strategies: from basic science to clinical application.” J. of Pathology. 208:299-318.

Standard Operating Procedures

Acknowledgement Page

I, _____, have read the SOP for working with _____ Viral Vector. The following people will be conducting experiments using these vectors. The staff members know where to find a copy of this SOP in the laboratory and they understand the hazards and safe work practices as detailed therein.

Name	Job Title	Initials

Principal Investigator (print): _____

Principal Investigator (signature): _____