Adeno-Associated Viral Vectors

Background

Adeno-associated viruses (AAV) are single strand DNA viruses that require a helper virus (typically adenovirus, herpesvirus and vaccinia) to replicate. When there is no helper virus, AAV can insert its DNA into the host chromosome stably, preferably human chromosome 19, and remain latent. These viruses are infectious to humans with no known disease association.

AAV vector characteristics include:
- A limited cloning capacity (~4.5 kb)
- 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

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.

Host Range

Recombinant AAV vectors infect a wide range of mammalian cells.

Stability

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 stability of the protein capsid, these viruses are stable for up to a month at room temperature.

Approvals

Experiments using AAV require local IBC approvals before initiation of experiments.

Test Methods for Recombinant Virus-QC Tests

**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.
Adeno-Associated Viral Vectors: Standard Operating Procedures

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.

2. A certified Class II biosafety cabinet 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

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.

6. All laboratory staff working with or supervising work with AAV must be up to date with laboratory safety and bloodborne pathogens/biosafety trainings.

7. Signs and labels (including the universal biohazard symbol) must be placed to indicate each area where AAV is used or stored (including biosafety cabinets, incubators, refrigerators, laboratory entrance doors, etc.)

Personal Protective Equipment

1. Disposable gloves.

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

3. Goggles and/or face shield.

4. All work and manipulations of AAV must be conducted in a certified Class II biological safety cabinet. If there are extenuating circumstances or a biosafety cabinet is unavailable, please contact REHS (at the numbers listed at the end of this SOP) as additional precautions may be required.
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 Emergency Response Guide flip chart posted in the lab 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 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 Emergency Response Guide flip chart posted in the lab for after-hours exposure. 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 incident report through https://MyREHS.rutgers.edu to document the event.

Decontamination

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

◆ To make this solution, dilute 1 part Clorox to 5 parts tap water.
◆ Ensure a 15 minute contact time.
◆ Use this disinfectant for treatment of reusable equipment, surfaces, and liquid waste (final volume 1% 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.

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. A certified Class II biosafety cabinet must be used to change out rodent cages. After all animals are removed from their primary enclosure immediately autoclave or treat with chemical disinfectant. After disinfection, dump the cage contents and begin cleaning the cage for re-use. 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 in the animal room. EOHSS 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 the hazardous materials are to be used in the protocol. See the following example: “The protocol will be conducted at Biosafety Level 2 (BSL2) and Animal Biosafety Level 1 (ABSL1). Staff will use Class II biosafety cabinets for procedures that have the potential to generate aerosols, splash or spray. Personnel working on the protocol will be trained in the hazards of working with the recombinant adeno-associated virus and the measures they need to take to protect themselves. They will wear lab coats or gowns, protective gloves and protective eyewear for all aspects of the protocol.”

References


Adeno-Associated Viral Vectors: Standard Operating Procedures

Acknowledgement Page

I, ________________________________, have read the SOP for Adeno-Associated Viral Vectors. The following people will be conducting experiments using the AAV 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.

<table>
<thead>
<tr>
<th>Name</th>
<th>Job Title</th>
<th>Initials</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Principal Investigator (print): ________________________________

Principal Investigator (Signature): ________________________________

Contact Information:

Rutgers Environmental Health and Safety
27 Road 1, Building 4086
Phone 848-445-2550
Fax 732-445-3109
Website http://rehs.rutgers.edu
Protocols http://myrehs.rutgers.edu