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Adenoviral Vectors

Background

Adenoviruses are non-enveloped icosahedral viruses composed of a nucleocapsid and a double-stranded linear DNA genome. These viruses have been an effective vector for gene delivery due to its ease of production, genetic stability, and high transduction efficiency. Gene expression of adenoviruses and adenoviral vectors is transient due to the genetic material of adenoviruses not being incorporated into the host cell's genetic material, and thus is not replicated during cell division. Transmission of adenoviruses is primarily through respiratory droplets and fecal-oral routes transmission.

Adenovirus is a respiratory, mucous membrane, eye and gastrointestinal pathogen. Replication deficient as well as replication competent adenovirus can cause respiratory inflammation, corneal injury and conjunctival damage. This virus can remain infective even after chloroform and ether extractions. The replication deficient virus may be complemented *in vivo* – causing the vector to become replication competent.

The genome consists of four early transcriptional units, E1, E2, E3, and E4. The DNA is flanked by inverted terminal repeats (ITR), which promote DNA synthesis. To create an adenoviral vector particle, at least two items need to be present: the transfer vector and a packaging cell. The transfer vector will have deletions in the E gene to render the virus non-replicative. The packaging cell must provide the essential E1 gene in *trans* during vector packaging. Cell lines such as HEK 293 and PER.C6 contain this gene. A safety concern is that while these packaging cells are required to create the virus, there is a possibility that the viral vector can acquire the E1 gene from the cell genome through recombination, thus restoring the replicative ability to the virus.

First-generation adenoviral vectors contain a deletion of the regulatory E1 and E3 genes. Second generation vectors have deletions of E2 and E4, in addition to the same deletions as first-generation. Third-generation vectors, also called "gutless", have almost the entire genome deleted except the 5' and 3' ITRs and packaging signal. This allows for a large gene of interest to be inserted (up to ~35kb). However, it requires a helper virus to add the essential proteins into the host cell for production of the adenoviral vector.

Symptoms of Exposure

Exposure to adenovirus may cause acute respiratory illness (cold like symptoms), pneumonia, conjunctival infection (pink eye) or corneal damage.

Environmental Stability

Due to being non-enveloped viruses, adenoviral vectors are relatively stable and resistant to dehydration. Alcohol-based disinfectants are NOT effective. Adenoviral vectors can survive up to 8 weeks on work surfaces at room temperature. A broad spectrum disinfectant, such as sodium hypochlorite (i.e. 1:10 bleach solution) must be used to inactivate adenovirus and adenoviral vectors.

Containment Level

Adenovirus may be transmitted by aerosol, droplet and injection routes of transmission. Generally, adenovirus is classified as a Biosafety Level 2 (BSL-2) organism. Adenoviral vectors may be regulated at varying biosafety levels. At the discretion of the IBC, experiments may need to be conducted at Biosafety Level 3 (BSL3). In the IBC application, the PI must justify that the gene to be expressed is not particularly harmful, and include citations to support these statements.

Approvals

Experiments using adenovirus 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.

Viral vector stocks must be checked for recombinant virus using the E1a assay prior to use in vitro or in vivo. The vector stock should be tested at a limit of sensitivity of 1 in 10^6 virus particles compared to a known positive control (Zhang et al, 1995).

Laboratory Practices

1. No work with Adenovirus is permitted on the open bench.

2. A certified Class II biosafety cabinet 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 adenovirus 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 adenovirus must be current on their laboratory and bloodborne pathogens/biosafety training requirements.

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

8. All work and manipulations of Adenovirus must be conducted in a certified Class II biological safety cabinet. If there are extenuating circumstances or this biosafety cabinet is unavailable, 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 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 emergency operations plan at emergency.rutgers.edu. Your supervisor should submit an accident report at http://myrehs.rutgers.edu.

- **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 operations plan for further instructions. Your supervisor should submit an accident report at http://myrehs.rutgers.edu.

- **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. Your supervisor should submit an accident report at http://myrehs.rutgers.edu.

Decontamination

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

- To make this solution, dilute 1 part bleach to 9 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 Facility Requirements:

1. 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..

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

3. Special training must be given to all animal husbandry personnel on adenovirus, the hazards associated with the work, required practices and procedures and proper handling of bedding, cage washing, and all other husbandry materials associated with the experiment. Animal facility staff may provide this training in consultation with REHS.

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. Replication deficient Adenovirus)
   - 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

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

MSDS Health Canada http://www.phac-aspc.gc.ca/msdsftss/msds3e.html

NCI-Fredrick Safetygram (ISM-193, April 2001): http://web.ncifcrf.gov/Campus/safety/safetygram/ism-193.pdf

Stanford University, “Working with Viral Vectors,” https://ehs.stanford.edu/topic/biosafety-biosecurity/viral-vectors

University of Kentucky Adenovirus Fact Sheet: http://ehs.uky.edu/biosafety/adenovirus.html

University of Texas Health Science Center at Houston “Guidelines for the Safe Handling of Adenoviral Vectors in Laboratory, Animal and Human Experiments” http://www.uth.tmc.edu/safety/biosafety/adenovirus.htm

Zhang WW, Kock, PE, Roth, JA. 1995. “Detection of wild-type contamination in a recombinant adenoviral preparation by PCR.” Biotechniques. 18:444-447.

Campagna et al. 2016. “Factors in the Selection of Surface Disinfectants for Use in a Laboratory Animal Setting” https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4783637/

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):_____