



Adenovirus/Adenoviral Vectors

Background

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.

Symptoms of Exposure

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

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 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.

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 10⁶ 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.

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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.).

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 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 EOHSS (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 Emergency Response Guide flip chart posted in the lab for further instructions. The supervisor should submit an to UMDNJ Risk and Claims 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](#) 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 EOHSS 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](#) to document the event.

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♦ **EMERGENT EXPOSURES**

For situations in which exposure to adenovirus occurred and medical treatment is an emergency, personnel should report to the Emergency Room, and ensure their supervisor completes an [incident report](#) to document the event.

Decontamination

1. The most effective disinfectant against adenovirus 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).
2. Disinfectant alternatives include 2% glutaraldehyde, and 0.25% sodium dodecyl sulfate (SDS).
3. 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

Work under the assumption that animals may shed the recombinant adenovirus, and take appropriate precautions as described in this section.

1. When animals are infected with adenovirus/adenoviral vectors, an Animal Biosafety Level - 2 (ABSL-2) 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).
2. Infected animals may excrete adenovirus (especially in the first 72 hours after infection). Animals treated with the replication-deficient recombinant adenovirus should be housed in barrier cages in an ABSL2 room when possible. Otherwise all animals administered adenovirus must be isolated.
3. Rodents must be in closed caging i.e. with filter-top bonnets or ventilated caging and must have enough food and water after adenoviral administration so that husbandry and investigatory staff need not open a cage for 72 hours. For example, only as much as 400-grams or less of rats (up to one 400-gram rat or four 100-gram rats) can be permitted in a standard rat cage without a cage change for 72 hours).
4. Use a Class II biosafety cabinet or HEPA-filtered change-out hood (up to date on inspection) when opening rodent cages.
5. Large animal precautions must be implemented: minimize or eliminate aerosol producing manipulations, use N95 respirators in addition to required ABSL2 Personal Protection Equipment for remaining aerosol producing procedures, strictly disinfect entire room upon completion of experiments, use negative pressure rooms whenever possible.

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6. All bedding, waste and animals shall be treated as if they are contaminated with recombinant adenovirus. 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.
7. Animal carcasses must be placed in autoclave bags and be designated for infectious waste disposal.
8. 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 EOHSS.
9. All necropsies must be performed in a designated room using animal BSL-2 practices and procedures.
10. 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., Replication deficient Ad-5)
 - 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 2 (ABSL2). 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 adenovirus 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

CDC-BMBL, 5th ed., www.cdc.gov/od/ohs/biosfty/bmb15/BMBL_5th_Edition.pdf

Hazardous and Radioactive Waste Disposal Standard Operating Procedure, Comparative Medicine Resources <http://njms.umdj.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,” http://www.stanford.edu/dept/EHS/prod/researchlab/bio/docs/Working_with_Viral_Vectors.pdf

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.

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Acknowledgement Page

I, _____, have read the SOP for Adenovirus/Adenoviral Vectors. The following people will be conducting experiments using the Adenovirus/Adenoviral 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): _____

Contact Information:



Newark Campus
(973) 972-4812 ♦ Fax (973) 972-3694

Piscataway/New Brunswick Campus
(732) 235-4058 ♦ Fax (732) 235-5270

Scotch Plains Campus
(908) 889-2486 ♦ Fax (908) 889-2496

Camden/Stratford Campus
(856) 566-6189 ♦ Fax (856) 566-6352

Website ♦ <http://www2.umdnj.edu/eohssweb>