

	Environmental Health and Safety Standard Operating Procedure (SOP) - Working Safely with <i>Adenoviral</i> Vectors
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1. Introduction

Adenoviral vectors are based on the Adenovirus which is a pathogen of the respiratory and gastrointestinal tracts as well as the eye and mucous membranes. Symptoms of exposure include acute respiratory illness, pneumonia, conjunctival infection, and corneal inflammation leading up to scarification. *Adenoviral* vectors can infect a wide variety of cell types, including nondividing cells such as hepatocytes, and can be grown to high titers. *Adenoviral* vectors have been modified to provide a safer version of the Adenovirus in which the viral gene coding sequences have been deleted. These vectors offer a number of advantages; however, the need for a helper virus in their production requires that special precautions be taken to ensure that the presence of helper virus is minimized in the preparation of high-titer virus stocks. Both replication competent and deficient vectors can cause corneal and conjunctival damage. In addition, the replication-deficient virus may be complimented in vivo thereby causing the vector to become replication competent.

2. Modes of Transmission

Adenovirus may be transmitted by:

- a. Droplets,
- b. Aerosols, and
- c. Injection

3. Containment Level

Work with *Adenoviral* vectors must be conducted utilizing Biological Safety Level 2 (BSL-2) practices and procedures as identified by Carnegie Mellon University's Institutional Biological Safety Committee (IBC).

4. Approval

Experiments using *Adenoviral* vectors require the approval of the IBC before initiation.

5. Facility Considerations

The Principal Investigator must designate a laboratory that fulfills the facility requirements as outlined in the CDC/NIH publication [Biosafety in Microbiological and Biomedical Laboratories for Biological Safety Level 2 Laboratories](#). It is preferable that this be an inner lab with two doors between the Biological Safety Cabinet and the hallway. Air must flow from the hallway to this lab (negative to the hallway) and all air is exhausted outside the building, not recirculated. Environmental Health and Safety (EHS) can evaluate the negative pressure status of the laboratory.

6. Engineering Controls

The following safety equipment must be used when working with *Adenoviral* vectors:

- a. Certified Class II Biological Safety Cabinets,
- b. Sealed centrifuge rotors and/or safety cups, and
- c. Vacuum lines equipped with an in-line HEPA filter as well as a primary and secondary vacuum flask containing a 10% bleach solution.

7. Administrative Controls

Work with *Adenoviral* vectors should only be carried out by trained personnel and all personnel must be directed by a competent scientist. Access to the laboratory must be limited when the agent is in use. The laboratory must be posted with Carnegie Mellon University's Biohazard signage. Standard Operating Procedures (SOP's) for the planned procedures must be written and shall be present in the laboratory at all times. All staff involved with the handling and administration of adenoviral vectors should receive Biosafety training that covers safety procedures. It is the Principal Investigator's responsibility to identify the staff requiring this training, and to call the Biosafety office to schedule a training session.

8. Personal Protective Equipment

The following personal protective equipment **MUST** be worn when working with *Adenoviral* vectors:

- a. Gloves,
- b. Lab Coat,
- c. Goggles,
- d. Face shield, and
- e. For work that is conducted outside of the biological safety cabinet, respiratory protection should be worn. If you have any questions, contact safety@andrew.cmu.edu.

9. Special Handling Procedures

- a. Cells exposed to *Adenoviral* vectors may not be removed from the laboratory for experimental purposes unless inactivated by approved procedures.
- b. If you need to aerate cultures, it must be done slowly and in a manner that minimizes the potential for aerosol creation. This action must be carried out in a class II biological safety cabinet.
- c. When pouring and pipetting samples, it must be done gently and slowly and must be carried out in a class II biological safety cabinet.
- d. For Aspiration- use a plastic vacuum flask with a second vacuum flask connected to it as a backup, with non-collapsible tubing capable of withstanding disinfection. To the second vacuum flask attach a hydrophobic and a HEPA filter (or combination filter) to ensure that nothing is sucked into the house vacuum system. These 3 items must be attached in series from the vacuum source in the hood or a vacuum pump.

10. Decontamination/Clean-Up Procedures

All materials that have come into contact with *Adenoviral* vectors should be disinfected using a 1:10 bleach (or appropriate alternative) solution before disposal. Additionally, all work surfaces must be disinfected with a 1:10 solution of bleach (or appropriate alternative) once work is completed and at the end of the work day. (Note: A 15 minute contact time is required for decontamination) Note: Alcohol solutions MUST NOT be used for decontamination!

11. Waste Disposal Procedures

- a. Non-Sharp Waste- All cultures, stocks, and cell culture materials must be disinfected and autoclaved (for one hour at 121 degrees centigrade) prior to being disposed of into a double red bag-lined biohazard box.
- b. Sharps Waste-All needles, syringes, razors, scalpels, Pasteur pipettes and pipette tips must be disposed of in an approved, puncture resistant sharps container. Sharps containers must not filled more than 2/3 of their capacity.

12. Injury/Exposure Incident Procedures

- a. Eye or Mucous Membrane Exposure from Splash or Aerosols- rinse a minimum of 15 minutes using eye wash and report the incident to your supervisor immediately.
- b. Skin Contamination-Wash affected areas with soap and water for 15 minutes and report the incident to your supervisor immediately.
- c. Needlestick and/or Sharps Exposure- Wash affected areas with soap and water for 15 minutes. Immediately notify your supervisor. Your supervisor will complete the Post-Exposure Incident Report and submit it to EHS within 24 hours of the exposure. Contact University Police at 8-2323 to arrange for appropriate medical attention.

13. Spill Response Procedures

The following steps must be taken when cleaning up a spill:

- a. Stop, notify others and isolate the area.
- b. Put on appropriate PPE (lab coat, gloves, eye and face protection).
- c. Remove glass/lumps with forceps or scoop if applicable and place into a rigid, puncture resistant container.
- d. Small spills-Place paper towels soaked in bleach directly on the spill and let soak for 20 minutes.
- e. Wipe up area and discard towels in biohazard waste container.
- f. Continue wiping area with paper towels soaked in bleach until the spill area is completely cleaned.
- g. Discard all materials in biohazard waste container.
- h. Wash hands thoroughly.

14. References

- a. Biological Safety Principles and Practices, 3rd edition, 2000. ASM Press. Edited by Diane O. Fleming, Ph.D, and Debra Hunt, Dr.P.H.

- b. [*Biosafety in Microbiology and Biomedical Laboratories*, 6th edition](#), December 2024. Centers for Disease Control.
- c. [*Canadian Laboratory Centre for Disease Control Material Safety Data Sheets*](#).
- d. [*Guidelines for Research Involving Recombinant DNA Molecules*](#), April 2024. National Institutes of Health.
- e. *Guidelines for the Safe Handling of Replication Defective Recombinant Adenoviral Vectors*. Bruce Trepannell, M.D.; edited by Ba-Bie Teng, Ph.D.
- f. *Minimum Facilities and Safety Practices Required for Use of Adenovirus Vectors in Laboratory and Animal Experiments*. MD Anderson Safety.

15. Revisions

Date	Documented Changes	Initials
10/2019		
1/14/2021	Updated Format and Accessibility Update	MAS
1/18/2024	Reviewed – no updates necessary	AJL
3/11/2025	Update links for BMBL and NIH Guidelines	MAS