

Tel-Aviv University –Safety Unit

Standard Operating Procedure for Working with Adenoviral Vectors in Animals

This document applies to cases, which the safety unit defines as **BSL-2**

1. Health hazards

Adenoviruses (members of the family Adenoviridae) are medium-sized (90–100 nm), nonenveloped viruses, with an icosahedral capsid at 70-90 nm in diameter, each contains a single linear, double-stranded DNA genome of approximately 36 kb. The adenoviruses replication cycle occurs in the cell nucleus, but the genetic material of the adenoviruses is not incorporated into the host cell's genetic material and is not replicated during cell division, providing transient expression.

The Adenoviruses have a broad range of vertebrate hosts; in humans, 57 distinct adenoviral serotypes have been found to cause a wide range of illnesses, from mild respiratory infections in young children to life-threatening multi-organ disease in people with a weakened immune system.

Adenovirus is a major agent of acute respiratory disease, mainly caused by serotypes 4 and 7, and is characterized by fever, rhinitis, pharyngitis, cough, and conjunctivitis. Other common illnesses can be observed in the respiratory tract, gastrointestinal tract, and eyes (acute follicular conjunctivitis).

Host Range: Humans and animals. Experimentally, human adenovirus can infect virtually all mammalian species, including monkeys, cotton rats, rabbits, and rodents.

Zoonosis: non

Infective Dose: Inhalation of as few as 5 adenovirus particles can cause disease in susceptible individuals. The incubation period is approximately 2 to 14 days.

Mode of Transmission: Respiratory (adenoviruses are primarily spread via respiratory droplets) and fecal-oral routes. Infection can also spread through contaminated fomites, fingers, ophthalmic solutions, and airborne particulates.

Infected/transduced cells : A wide range of human and non-human cell lines cells (dividing and non-dividing).

vector systems

Vector information: Adenoviral vectors are based on the Adenovirus.

Adenoviral vectors can infect a wide variety of cell types, including non-dividing cells such as hepatocytes, and can be grown to high titers.

Both replication competent and deficient vectors can cause corneal and conjunctival damage (therefore, goggles should be worn when working with the vector). In addition, the replication-deficient virus may be complemented in vivo thereby causing the vector to become replication competent.

Reduce risk of exposure by reducing potential of replication competent adenovirus vector is achieved by using one of the following methods:

- * 1st generation vector – deletion of E1 or E1/E3
- * 2nd generation vector – deletion of E1, E3, E4
- * 3rd generation vector – all viral gene deleted; only essential cis-acting sequences retained.

<p>2. Housing and Biosafety consideration</p>	<p>The work must be done under ABSL-2 containment in the following cases:</p> <ol style="list-style-type: none"> 1. When a known helper virus is present or the host animal may potentially contain virus that could act as a helper (e.g. mice replete with retroviruses). 2. For recombinant Adenovirus : Because residual helper virus may not be completely inactivated during ADENOVIRUS purification ,helper virus may be present . 3. When Adenovirus vector expressing highly biologically active molecules such as oncogenes (including siRNA to a tumor suppressor) allergens or cytokines. <p>The NIH classifies the following ADENOVIRUS vectors as BSL-1:</p> <ol style="list-style-type: none"> a. adeno-associated virus (ADENOVIRUS) types 1 through 4 b. recombinant ADENOVIRUS constructs, in which the transgene does not encode either a potentially tumorigenic gene product (oncogene) or a toxin molecule c. also are produced in the absence of a helper virus.
<p>3. Training</p>	<p>Practical experience with animal care/maintenance, as well as general biosafety, is required.</p>
<p>4. Personal Protective Equipment (PPE)</p>	<p>Gloves (consider double-gloving), Eyes safety goggles, Lab coat, disposable shoe covers and Animal handling gown.</p> <p>N-99 respirator mask covering the mouth and nose when not working in a Class II Biosafety Cabinet (BSC).</p> <p>Appropriate PPE should also be used for lower arms such as sleeve covers or securing gloves over the sleeves of laboratory coat.</p> <p>Exposure risks: Transmission of adenovirus vectors can occur through inhalation of aerosolized droplets, mucous membrane contact, ingestion and accidental injection.</p> <p>When handling adenovirus-containing cultures outside of containment equipment, a respirator (N99 mask) should be worn.</p> <p>Combination of goggles and respirator provided adequate protection (mucosal and respiratory).</p> <p><i>Personnel should not work with adenovirus vectors, if skin is cut or scratched.</i></p>
<p>5. General . Precautions for Animal Use</p>	<p>Tools (as, syringe, blades and safety needles where possible) should be adapted for BSL-2. Have a sharps container in close vicinity.</p> <p>Animals should be restrained or anesthetized during injection.</p>
<p>6. Environmental / Ventilation Controls</p>	<p>Work should be conducted in ABSL-2 facility, over absorbent pads in a class II type A1 or A2 biological cabinet.</p> <p>Survival Outside Host: Most serotypes are stable at 36 °C for a week, for several weeks at room temperature, and for several months at 4 °C.</p> <p>Adenoviruses are very stable in the environment and persist for 7 days to 3 months on dry inanimate surfaces.</p> <p>They can also survive for weeks in tap water, sewage effluent and sea water.</p> <p>Adenovirus type 2 can survive on common environmental surfaces for up to 8 weeks at room temperature.</p> <p>The following safety equipment MUST be used when working with Adenoviral vectors:</p> <ul style="list-style-type: none"> •Certified Class II Biological Safety Cabinets •Sealed centrifuge rotors and/or safety cups

	<ul style="list-style-type: none"> •Vacuum lines equipped with an in-line HEPA filter as well as a primary and secondary vacuum flask containing a 10% bleach solution.
<p>7. Animal handling practices</p>	<p>Mice are permissive host for the viral vector.</p> <ol style="list-style-type: none"> 1. Animals must be housed in filter top cages marked as biohazards (including the name of the pathogen/biohazard and date of administration). Handling the cages (including bedding) will be done only by the researchers. 2. Use a class II Biological Safety Cabinet at all times (especially during injection or any surgical procedure), when performing work on these animals and/or when moving animals from dirty to clean cages. 3. Infected animals may shed Adenovirus for the first three days after treatment; take precautions to avoid the creation of aerosols when changing or washing cages, or cleaning the room. 4. Dead animals must be placed in primary plastic bags, which are then placed in biosafety bags for infectious waste incineration. 5. All surfaces and racks that may be contaminated will be decontaminated with 1% bleach or virusolve ASAP. 6. When changing cages, use a standard microisolator technique: place the cage containing the animals, under the biological safety cabinet and transfer the animals into a clean cage. spray the dirty cage with virusolve, remove from the safety cabinet and place on a transfer rack . when all cages have been changed, spray the dirty cages and rack again with virusolve, and cover the rack. Put on a pair of new gloves and bring the rack directly to the autoclave. immediately autoclave the dirty cages (1 hour at 121°C/250°F, 15psi of steam pressure). Once the autoclave cycle is completed, the cages can be emptied and the bedding disposed of in a normal fashion. **In cases where the use of autoclave (within the animal facility) is not an option: the cages (bedding) must be emptied inside the BSL-2 cabinet, directly to a double biohazard bags. Alternately : transport the bags of cages to a HEPA filtered dumping station that draws air away from the use (it is recommended to use a mask) or fume hood. Mucosal protection must be worn anytime contaminated materials/equipment is handled outside a BSC. **Before closing the bags, carefully, add a small amount of water (250ml) to improve the sterilization process. <i>Do not close the bag completely/tightly (in order to avoid entering of steam during the sterilization process).</i> Spray the dirty bag with 0.5% bleach or virusolve. Remove from the safety cabinet and place on a transfer rack/container. Put on a pair of new gloves and bring the rack/container, directly to the collection point of your department.
<p>8. Decontamination</p>	<p>Disinfection: 10% bleach (recommended). Adenoviruses are resistant to lipid disinfectants, but are inactivated by formaldehyde and chlorine. They can be inactivated by contact with 1:5 dilution of bleach.</p>

	<p>All materials that have come into contact with Adenoviral vectors should be disinfected using a 1:10 bleach solution before disposal. Additionally, all work surfaces must be disinfected with a 1:10 solution of bleach once work is completed and at the end of the work day. (Note: a 15 minute contact time is required for decontamination).</p> <p>Adenovirus can be inactivated by heat: heating to 56 °C for 30 min, 60 °C for 2 min, and autoclaving will destroy infectivity.</p> <p>Adenovirus, as well as other non-enveloped viruses, is quite resistant to alcohol disinfectants.</p>
<p>9. Spill and Accident Procedures</p>	<ol style="list-style-type: none"> 1. Evacuate area, remove contaminated PPE and allow agents to settle for a minimum of 30 minutes. Initiate spill response procedure. 2. Cover the spill with absorbent material. Starting at the edges and work towards the center. 3. Carefully pour disinfectant over the absorbed spill, again starting at the edges. Saturate the area with disinfectant. 4. Allow sufficient contact period to inactivate the material in the spill. Non-viscous spills require 15-20 minutes: viscous spills require 30 minutes. 5. Use paper towels to wipe up the spill, working from the edge to center. Use tongs or forceps to pick up broken plastics, glass or other sharps that could puncture gloves 6. Discard absorbent material in Chemical waste bags. 7. Clean the spill area with fresh paper towels soaked in disinfectant. Thoroughly wet the spill area, allow to disinfect for 15-20 minutes longer, and wipe with towels. 8. Discard all cleanup materials (soaked with disinfectant) in Chemical bag, and any contaminated PPE (pay special attention to gloves and shoe covers) in a biohazard bag. Close and secure the bags. 9. Place bag in a second biohazard bag, secure and disinfect by autoclaving. <p><u>Exposure:</u></p> <ol style="list-style-type: none"> 1. In case of skin contact or injection with Adenovirus vectors, wash the affected area with soap and water for at least 15 minutes. Consult with Employee Health Center. 2. For eye exposure, flush with water for at least 15 minutes. Consult with Employee Health Center. 3. Report incident to supervisor. Supervisor reports the accident/injury to the Biosafety Unit.
<p>10. Waste Disposal</p>	<p>Autoclave all waste (1 hour at 121°C/250 °F, 15psi of steam pressure).</p>
<p>I hereby confirm that I have read the SOP (Standard Operating Procedure) for Working with Adenovirus vectors in Animals, and agree to follow these procedures.</p>	
<p>Name:</p>	<p>Title:</p>
<p>Signature:</p>	<p>Date:</p>

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