

# **UTHHSC Guidelines for the Safe Handling of Adenoviral Vectors In Laboratory, Animal and Human Experiments**

## **Purpose**

The purpose of these guidelines is to provide clinical and molecular information about human adenoviruses and the vectors derived from them and to describe procedures for their safe handling during laboratory, animal and human experiments.

## **General Information on Human Adenovirus**

There are approximately 50 different serotypes of human adenoviruses. Some can induce a spectrum of illnesses including acute, self-limiting pharyngitis (a common cold), keratoconjunctivitis (pink eye), and diarrhea. In rare cases, human adenovirus may cause hepatitis (inflammation of the liver), or inflammation of other organs. Also, in rare situations when an individual is already seriously immunocompromised (has weakened defenses against infections, i.e. AIDS patients), the outcome could be a blood infection. However, in most instances, adenoviruses cause only a common upper respiratory illness known as a "cold." One of the most common adenovirus serotypes that infect the human respiratory tract is Adenovirus Type 5. This is the serotype that recombinant adenoviral vectors are currently derived from.

The epidemiology of adenovirus indicates that it is found worldwide with high occurrences in children under the age of 5 years. In tropical regions, adenovirus incidences usually occur in wetter areas, while temperate regions experience seasonal occurrences with the highest incidences occurring in the fall, winter, and early spring.

The mode of transmission of adenovirus is directly by oral contact or aerosolized droplet exposure to the mucous membrane with an incubation period of 1 to 10 days in healthy humans. There are no specific antiviral drugs currently available, but adenovirus shows susceptibility to chemical disinfectants such as 1% sodium hypochlorite and 2% glutaraldehyde.

A question is occasionally raised by individuals with regard to the potential problem of an investigator suffering from a cold while working with adenoviral vectors. Most adult individuals have had natural adenoviral infection early in childhood, which results in immunity to subsequent adenoviral infections. If Biosafety Level 2 precautions are strictly followed, there is a **very low** risk of self-contamination with the vector.

## **Recombinant Adenoviral Vectors**

Laboratory-made recombinant adenoviral vectors are derived from the type 5 adenovirus. Common deletions in adenoviral vectors include the E1 and E3 regions. The E1 deletion

renders the virus incapable of autonomously reproducing itself and the E3 deletion makes the virus more susceptible to the human immune defense system and also provides an area for transgene insertion. The E1 deletion is replaced with an "expression cassette" that consists of a promoter, research gene and poly A signal. The recombinant vector can be produced to very high titers in Human Embryonic Kidney (HEK) 293 cells (the titer can reach up to  $10^{12}$  infection units per millimeter).

There are several points that must be kept in mind with regard to recombinant vectors:

1. Despite the fact that humans are probably immune to this vector, a vector may still infect an individual if he/she is exposed to a high titer. Nonetheless, theoretically, recombinant adenoviral vectors would not replicate.
2. While exposure to wild type, replication competent adenovirus may be a low risk, the risk of exposure to recombinant adenoviral vectors is unknown. Moreover, the potential risk of exposure to different recombinant adenoviral vectors may not be the same. It is believed that some vectors may have minimum risk (e.g., null and LacZ vectors) while others (e.g., interleukin, TNF, or immune effector vectors) may pose a higher risk.

Based on the current understanding, recombinant adenoviral vectors have been classified as Class I (minimum risk) and Class II (potentially higher risk). The safety conditions under which these two classes are to be used are very similar, that is, generally all procedures are performed under Biosafety Level 2 (BSL-2), while the Class II vectors are performed under Biosafety Level 2 with the possible addition of Biosafety Level 3 practices and/or equipment.

The classification of adenoviral vectors into classes is only intended as a guide. Based on clinical data, the wildtype virus (adenovirus type 5) was categorized in the Class I classification. All other categorizations are less clear. The attenuated viruses with the E1 region deletions might naturally be expected to be less dangerous, due to their reduced capacity for autonomous viral replication, and categorized as Class I. Based on transgenic animal studies, vectors expressing transgenes such as markers (e.g., LacZ, neomycin phosphotransferase, and chloramphenicol acetyl transferase) are grouped in Class I. On the other hand, vectors expressing a product that is known to be toxic or involved in the regulation of cell growth should be grouped in Class II. As alluded from the above, if the recombinant vector being studied has minimal safety data, or there is concern over the over-expression of the transgene(s) or the location of the transgene(s) expressed, a conservative safety approach should be taken and the use of Biosafety Level 2+ practices should be used when appropriate.

### **Administrative Procedures Prior To Protocol Initiation**

- Prior to submitting requests to the Institutional Biosafety Committee (IBC), contact the Biological Safety Program of Environmental Health and Safety at 713-500-8100 to discuss biosafety issues and precautions.

- Write specific Standard Operating Procedures (SOP's) for the planned procedures, which will be included in the IBC submission.
- If animals and/or humans are to be used in the protocol, the Animal Welfare Committee (AWC) and/or the Committee for the Protection of Human Subjects (CPHS) will also have to grant approval of this protocol.
- If working with humans, include in your submission, (1) copies of the "Investigator's Brochure", (2) Section V from the corresponding FDA IND and (3) a copy of the documentation submitted to the CPHS.
- Designate the rooms where the adenovirus will be handled and the areas where post-inoculated animals will be housed. The Biological Safety Program will ensure that all rooms where virus administration and propagation is to take place are suitable.
- Modifications to the protocol including room, agent or personnel changes must be submitted in writing to Biological Safety for IBC review and update.
- All staff involved with the handling and administration of adenoviral vectors should receive Biosafety training that covers hazardous communication (HAZCOM) and safety procedures, before final IBC approval. It is the Principal Investigator's responsibility to identify the staff requiring this training, and to call the Environmental Health and Safety office to schedule a training session.
- Respiratory protection is required for staff involved with handling and administration of adenovirus vectors outside of containment equipment (i.e. biological safety cabinets). Fit testing must be completed before final IBC approval. It is the Principal Investigator's responsibility to identify the staff requiring fit testing.

### **Guidelines for the Safe Handling of Adenoviral Vectors in Laboratory, Tissue Culture, Animal and Human Experiments**

#### **Transport of Adenovirus:**

- Call the Biological Safety Program for assistance in transporting infectious materials at 713-500-8100.
- Transport all material in a double-sealed leakproof container.
- Label the container with a biohazard symbol, the name of the agent, the amount, and the Principal Investigator's name and telephone number.

#### **Laboratory Experiments:**

- Laboratory coats, gloves, and safety glasses or goggles must be worn.
- Materials containing adenovirus should be handled inside biological safety cabinets (Class IIA, IIB1, or IIB2), whenever possible.
- Wear a respirator (N95 NIOSH classification TC-84A) for which the wearer has been fit tested, to protect against exposures from aerosolization during spills of virus containing materials when handling adenovirus-containing cultures outside of containment equipment.
- When performing centrifugation procedures, use centrifuge safety cups.
- Protect the vacuum lines and system with disinfectant traps and filter.

- Pay special attention to the possible generation of aerosols from waste materials.

### **Tissue Culture Experiments:**

- Incorporate the above precautions.
- All tissue culture work should be conducted in a biological safety cabinet capable of producing both product and personnel protection (Class IIA, IIB1, or IIB2).
- A biological hazard sign indicating the use of adenovirus should be placed outside tissue culture room and on the biological safety cabinet.
- Laboratory coats used inside the tissue culture room should not be worn outside the tissue culture room.
- Materials should not be stored inside the biological safety cabinet (BSC). Take only what is needed to perform the procedure(s) and place it in the BSC upon initiation of the procedure. Upon conclusion of the procedure(s), remove everything from the BSC. This applies to all equipment with the exception of pipettes and a rack to hold the suction apparatus tubing which are allowed to be left in the BSC.
- Serological pipettes and pipette tips should be decontaminated in a virucide, such as a 1:10 dilution of household bleach (final concentration 0.525%), for at least 15 minutes prior to discarding in solid biohazard waste. For this purpose, a beaker containing a virucide may be kept inside the BSC while experimental procedures are being performed. If pipettes and pipette tips are to be re-used following decontamination, they should be rinsed in water prior to autoclaving.
- All plasticware placed inside the hood while working with the virus must be decontaminated with a virucide prior to autoclaving. This can be done by spraying all plasticware with a 1:10 dilution of household bleach (final concentration 0.525%).
- Upon conclusion of procedures in the BSC:
  1. If aspirated liquid waste is 2/3 full, aspirate a virucide through the suction tube so that the final concentration is appropriate, allow it to soak for at least 15 minutes, and empty entire contents down the drain.
  2. Spray all work surfaces with a virucide and then with 80% ethanol. Allow the surface to air dry.
  3. When solid biohazard waste bag is full, seal it with autoclave tape, and take the bag (double bagged and in secondary containment) to the autoclave room for sterilization (the laboratory room number should be on the bag). Replace double bag in the secondary container. Some personnel do not have access to an autoclave; call the Waste Hotline at 713-500-5837 for pickup of boxes of red bag waste. Do not overfill biohazard waste bags.

### **Small Animal Experiments**

(During wash-out period of adenovirus vector, ~ 10 days)

Assume the wash-out period for adenovirus to be a 10 day minimum unless it can be demonstrated on HEK 293 cells or other permissive cell lines to be different for an application. It is the responsibility of the investigator to submit any viral shedding results to the Biological Safety Program and veterinarians at the Center for Laboratory and Animal Medicine Care.

- Incorporate the above laboratory experiment precautions.
- Perform all administrations and manipulations of animals inside of BSC (Class I, IIA, IIB1 or IIB2).
- Use microisolators or containment type animal housing.
- Perform cage change-outs in BSC (Class I or II).
- Perform cage dumping in BSC's (Class I or II) or in negative pressure dumping stations installed inside of Class I or II BSC's, or autoclave the bedding before discarding in a conventional manner.
- Disinfect cages by spraying with a virucidal solution inside of a BSC (Class I or II), before removing from the BSC.
- Spray cage racks with a virucidal solution before removal from a BSL-2 facility. Allow to air dry.

### **Large Animal Operations and All Operations That Can Not Be Performed Inside Of Appropriate Containment Equipment**

Assume the wash-out period for adenovirus to be a 10 day minimum unless it can be demonstrated on HEK 293 cells or other permissive cell lines to be different for an application. It is the responsibility of the investigator to submit any viral shedding results to the Biological Safety Program and veterinarians at the Center for Laboratory and Animal Medicine Care. Work at a minimum of BSL-2 facilities and use BSL-3 practices with special attention paid to the generation of aerosols.

- Where practical incorporate the precautions stated above from laboratory and small animal precautions. However, also include additional precautions to contain aerosol generation. These precautions are necessary when the procedures or animals can not be contained inside a Class I or II BSC.
- Work only in negative pressure (exhausted) rooms that have at least 6 ACH (air changes per hour) or HEPA (high efficiency particulate air filter) filtration prior to recirculation of room air. Environmental Health and Safety must grant all exceptions to this requirement.
- Personnel entering large animal housing rooms must wear a half-mask HEPA filtered respirator or a N95 respirator (N95 NIOSH classification TC-84A) for which the wearer has been fit-tested. A dust/mist mask is acceptable if not administering a concentrated viral preparation and the time in the room is expected to be 15 minutes or less.
- Review and write a specific SOP for each particular experiment design or trial. Incorporate specifics about personal protective equipment and disposal methods of the materials used in the procedures. Address post-experiment room cleaning procedures and disinfection/disposal of contaminated materials in the SOP.
- Dispose of waste material at the site of use by placing it in a biohazard box that is sealed and then incinerated.

## **Human Experiments:**

In Pharmacy: Work in BSL-2 facilities with BSL-2 practices with special and specific attention paid to the generation of aerosols as specified below.

- Laboratory coats, gloves, and safety glasses or goggles must be worn.
- Materials containing adenovirus should be handled inside biological safety cabinets (Class IIA, IIB1, or IIB2), whenever possible.
- Wear a respirator (N95 NIOSH classification TC-84A) for which the wearer has been fit tested, to protect against exposures from aerosolization during spills of virus containing materials when handling adenovirus-containing cultures outside of containment equipment.
- Protect the vacuum lines and system with disinfectant traps and filter.
- Pay special attention to the possible generation of aerosols from waste materials.
- Equipment, waste, and spills will be decontaminated with a final concentration of 0.525% household bleach.

## **Administration of Concentrated Adenovirus in Humans**

(Greater than  $10^9$  viral vectors)

- Perform all administrations involving concentrated materials in negatively pressured (exhausted) rooms that have at least 6 air changes per hour or HEPA filtration prior to recirculation of the room air. The Biological Safety Program must grant all exceptions to this requirement.
- The doors of the room must remain closed during administrations.
- Wear appropriate outerwear or gowns that are laundered by the institutional laundry service.
- Follow good infection control practices by not wearing contaminated outerwear or personal protective equipment while tending to other patients.
- Wear gloves and wash your hands after removing them and before exiting the room.
- Avoid touching your face with gloved hands.
- Wear safety glasses or goggles.
- Wear a dust/mist mask 95% efficient respirator, (N95 NIOSH classification TC-84A) for which the wearer has been fit tested, to protect against possible exposures from aerosolization of virus containing materials when handling adenovirus-containing vessels. Alternatively, a HEPA respirator (99.99% efficient NIOSH classification TC-21C) may be worn if the wearer has been fit tested.
- Pay special attention to the possible generation of aerosols from both procedures and waste materials.
- Employ Universal Precautions when handling all clinical specimens or body fluids.
- Disinfect all surfaces and equipment that come into contact with the adenovirus promptly, with an appropriate virucide.
- Have a written spill clean-up procedure in place and ensure that the materials for the procedure are available in the room.

- Package all biological waste in disposable biohazard boxes, which will be disposed of by contacting the Environmental Protection Program at 713-500-5837.
- Comply with any additional precautions stipulated by the IBC and CPHS.

## **Post Administration Patient Care**

### Inpatient

- Assume the wash-out period for adenovirus to be a 10 day minimum unless it can be demonstrated on HEK 293 cells or other permissive cell lines to be different for an application.
- Place patient in a separate negative pressure room or demistifier tent if available. If separate waiting/examination room is unavailable or if patient requires transportation to ancillary departments, a respirator should be worn by the patient.
- Schedule patient to minimize exposure to other patients.
- Upon dismissal, if such takes place within 3 days of the last vector administration, counsel and instruct patient as per Outpatient criteria listed below.
- Comply with any additional precautions stipulated by the IBC and CPHS upon approval.

### Outpatient

- The outpatient should be counseled prior to release, to avoid public areas, transportation, young children, the aged, and the immunosuppressed, for 3 days after the last adenoviral administration. If avoidance of these situations is not possible during this time period, the outpatient should be counseled to wear a respirator.
- Comply with any additional precautions stipulated by the IBC and CPHS upon approval.

## **Handling of Patient Samples and Sample Post Adenovirus Administration**

BSL-2 practices with special and specific attention paid to the generation of aerosols as specified below:

- Materials containing adenovirus should be handled inside biological safety cabinets (Class IIA, IIB1, or IIB2), whenever possible.
- Protect vacuum lines and system with disinfectant traps and filters.
- Wear a respirator (N95 NIOSH classification TC-84A) for which the wearer has been fit tested, to protect against exposures from aerosolization during spills of virus containing materials when handling adenovirus-containing cultures outside of containment equipment.
- Use Universal Precautions.
- Wear safety glasses/goggles to protect against splashes and spills.
- Wear gloves and wash your hands after removing them and before exiting the room.
- Avoid touching your face with your gloved hands.
- Pay special attention to the possible generation of aerosols from waste materials.

- Propagation of viable adenoviral vector from clinical material should be conducted exclusively at BSL-2 (facilities and BSL-2 practices).
- Comply with any additional precautions stipulated by the IBC and CPHS upon approval.

## **References**

*Minimum Facilities and Safety Practices Required for Use of Adenovirus Vectors in Laboratory and Animal Experiments.* MD Anderson Safety.

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*Biosafety in Microbiology and Biomedical Laboratories,* 4th edition, May 1999. Centers for Disease Control. [www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4.toc.htm](http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4.toc.htm)

Canadian Laboratory Centre for Disease Control Material Safety Data Sheets. [www.hc-sc.gc.ca/main/lcdc/web/biosaftey/msds/index.html](http://www.hc-sc.gc.ca/main/lcdc/web/biosaftey/msds/index.html)

All references are available from Environmental Health and Safety by calling 713-500-8100.