



Use of Human Adenovirus / Adenovirus Vectors Exposure Control Plan for Laboratories

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I. Applicability

This plan applies to all research labs engaged in research on human adenoviruses or making use of human adenovirus vectors, commercially obtained or otherwise. The plan also applies to all labs conducting research that involves contact with animals potentially infected with human adenoviruses, or animals into which recombinant adenoviruses have been introduced experimentally.

II. General Precautions

1) General risk assessment. Adenoviruses are pathogens of the respiratory and gastrointestinal mucous and ocular membranes. The virus does not have to be replication-competent to cause corneal and conjunctival damage. Goggles or side-splash protective safety glasses must be worn when working with the virus or viral vectors.

2) Complementation. The recombinant adenoviruses made with expression system kits are defective viruses that are deleted in the E1 and E3 regions. They will not normally replicate in cells other than those which complement *in trans* the E1/E3 proteins, normally 293 cells. Replication-defective vectors or rDNA constructs may also be complemented *in vivo* by wild strains of adenovirus, which continually circulate among humans.

3) Replication competence. Replication competent adenoviruses (RCA) do occur in viral stocks of replication defective adenoviruses. These RCA emerge as a result of a double crossover between the homologous overlapping sequences present in the recombinant virus and the 293 genome. This crossover results in the loss of the insert gene and its replacement by the E1 region, making the recombinant virus replication competent. Adenovirus stocks contain increasing amounts of RCA after increasing numbers of passages in 293 cells. Thus, to avoid the accumulation of RCA in viral stocks, always plaque purify the adenoviral stocks to be amplified, and use low-passage number 293 cells. Passage of the 293 cells beyond four passages increases the number of RCA that emerge.

4) Virus purification / concentration. For use in animals, purification of adenoviruses with CsCl gradients or by pelleting the virus through a cushion of CsCl is necessary to remove defective particles and media contaminants. Cell lysates contain large amounts

of fiber and penton proteins which are known to be cytotoxic, media, serum and cellular debris. These contaminants elicit a very strong immune response in the host animal. Handling of concentrated adenovirus requires special precautions, particularly in minimizing aerosols. If procedures involving the formation of aerosols using concentrated virus preparations are required, they should be conducted inside a biological safety cabinet.

III. Laboratory Practices

Requirements:

- 1) **General Lab Practices.** BSL-2 practices will be used at all times. Eating, drinking, smoking, applying cosmetics or lip balm, and handling contact lenses are prohibited in work areas where there is a reasonable possibility of exposure to pathogens. All procedures involving cultures, blood or other potentially infectious materials shall be performed in such a manner as to minimize splashing, spraying, spattering and the production of droplets. Mouth pipetting is prohibited. No food or drink will be stored in refrigerators, freezers, shelves, cabinets or on counter tops or benches within the laboratory or other areas where infectious materials may be present. Garments, gloves or other personal protective equipment shall be removed and replaced immediately or as soon as feasible if they become contaminated with infectious materials. *All personal protective equipment is removed before leaving the laboratory.*
- 2) **Vacuum Lines.** All vacuum lines must be fitted with a HEPA filter (for example, the Vacushield™ in-line hydrophobic filter from Gelman Science).
- 3) **Manipulations.** No open work with adenoviruses is permitted on the bench. A biological safety cabinet must be used for all manipulations such as pipetting, harvesting cells, collection of viral supernatants, and opening / closing of centrifuge tubes.
- 4) **Centrifugation.** Centrifugations must be done in tight closing tubes inside sealed rotors or within safety cups. Microfuges may only be used inside a biological safety cabinet when virus preparations are being subjected to centrifugation. Pressurized or vacuum containers should be covered during opening or when needles are removed from pressurized vials. Use gauze that has been soaked with alcohol or cut-out squares absorbent lab matting. If the material in the container is infectious or potentially infectious, defective virus or otherwise, such activities shall be done *only* within the BSC.
- 5) **Hand-washing.** Frequent hand-washing will be practiced whenever the hands become visibly contaminated with material, after the completion of work tasks involving the handling of infectious materials, before leaving the laboratory, and after removing gloves. After exiting the lab, hands should be again washed before eating or handling contact lenses. Strict adherence to hand-washing practice will prevent contact transfer to mucous membranes of infectious agents. If cuts, scrapes or other lesions are present on the skin, gloves must be worn all times to prevent contamination of the non-intact skin. Workers with skin lesions or dermatitis on hands or wrists will not perform procedures with

potentially infectious materials even if they are wearing gloves.

The proper procedure for hand-washing is as follows:

- a. Use a hands-free sink if possible. If not, open faucets to create a stream of warm running water.
- b. Wet hands under running warm water, then use soap. Preferably, anti-bacterial soap should be used. Hibiclens® soap is an excellent anti-microbial soap.
- c. Lather well beyond the wrists. Work all surfaces thoroughly including the wrists, palms, back of the hands, fingers and under the fingernails. Rub hands together for at least 15-20 seconds.
- d. Rinse thoroughly with clean water. Be sure not to touch the side of the sink.
- e. Dry hands completely. If a hands-free sink is not used, cover the faucet handle with a paper towel when turning off the water to protect your clean hands from pathogens that might be present on the handle.

6) Transport of specimens. Infectious substances and specimens shall be transported to other labs or areas using leak-proof containers within a secondary container to safely manage spills if they occur. Test tubes or other small samples should be transported within a rack placed within a secondary container (such as a modified tackle box) labeled with a biohazard symbol. Capillary tubes should be transported in a solid-walled secondary container, for example a plastic snap or screw-top tube. Transport of cultures or hemacytometers from the BSC to the microscope or other areas within the lab shall make use of trays or other secondary containers.

6) Routine Cleaning and Disinfection. Routine cleaning of work surfaces with disinfectant must be done after completion of each procedure and at the end of each work day, and additionally as necessary when spills occur. Disinfection of adenoviruses contaminated surfaces can be accomplished with most phenolics compounds or diluted bleach. When using bleach, dilute to 10% (v/v) with water and prepare fresh daily.

7) Spill Cleanup. Decontamination shall be done following spills of all infectious or potentially infectious materials. Cleanup of small spills involves the following steps:

- a. If broken glass is present, pick it up with tongs or other mechanical device.
- b. Flood the spill with an appropriate disinfectant. Potentially infectious material may be treated with an approximate one tenth volume of undiluted bleach to achieve a final 10% concentration. Absorb the spill with either paper towels or absorbent lab “diaper” material or granular material impregnated with disinfectant.
- c. Carefully scrape up the absorbent materials and discard in the biohazard waste.
- d. Clean the area with soap and water.
- e. Decontaminate with an appropriate fresh disinfectant.

8) Disposal of Wastes. Solid wastes shall be collected into biohazard bags suitable for autoclaving. Two layers of biohazard bags used for collection shall be placed inside a suitably sized leak-proof secondary container, such as a large plastic bucket fitted with a lid. When full but not overflowing, the bags are closed and autoclaved. If waste is to be removed to a common-use autoclaving facility, it should be transported to the facility while still inside a closed secondary container. *Never* attempt to manually compact solid infectious wastes. The bags are removed from the secondary container only when they are placed in the autoclave or into a leak-proof tub in preparation for autoclaving. Sharps containers are tightly closed and autoclaved.

Fluid wastes (such as culture supernatants) may be discarded after autoclaving by carefully pouring down the drain with water rinse. Bleach (10% final bleach volume) may be used as an alternative to autoclaving, but must be allowed to sit for at least 10 minutes before discard to the sanitary sewer.

9) Access. Laboratory doors shall remain closed at all times when work is in progress, and entry to the area is restricted. The principle investigator will establish specific written entry requirements and policies whereby only those individuals who have a need to enter and have been made aware of the potential hazards within are allowed access. Infectious materials stored within the lab, in freezers or refrigerators, should be secured in leak-proof double containers.

A biohazard warning sign must be posted at all entrances to the laboratory with:

- (1) the name of the infectious agent, "Human Adenovirus" (plus others, as used)
- (2) special requirements for entry, such as PPE requirements, and
- (3) name and phone number of the laboratory director or other responsible person.

IV. Personal Protective Equipment (PPE)

Requirements:

1) Gloves. Gloves are required whenever hand contact with potentially infectious materials is likely or anticipated. Gloves are also required to be worn when handling or touching contaminated items or surfaces and for performing vascular access procedures. Gloves must be worn when handling clinical specimens, infected animals or potentially contaminated equipment. In research laboratories, gloves should be worn for *all* procedures, cleaning spills, and handling wastes.

Gloves must *never* be washed or disinfected for re-use. Detergents, alcohol or other disinfectants may compromise the ability of the glove to resist penetration by infectious substances. Gloves must be changed when visibly contaminated, torn or whenever tasks are completed. All layers of gloves shall be removed before handling telephones, doorknobs or "clean" equipment.

Gloves are to be removed for discard "inside out" to keep the "dirty" side inward and thus prevent inadvertent contamination of laboratory surfaces or equipment. Hands are to

be washed with soap and warm water immediately after glove removal.

2) Protective Clothing. Shorts and sandals are not appropriate attire in an infectious disease laboratory due to the risk of exposure through exposed skin, and are not allowed. Laboratory coats, gowns or aprons are required at all times in the laboratory.

Lab coats, gowns or aprons must be removed prior to exit from the laboratory, and are either disposable or laundered on the premises. Alternatively, lab coats may be bagged and autoclaved, and then removed off-site for laundering. Plastic buttons may melt in the autoclave, so coats should be fitted with heat-resistant closures.

3) Respirator. A fit-tested NIOSH N-95 or N-100 respirator must be worn when working with concentrated virus preparations and performing aerosolizing procedures outside the BSC.

4) Eye protection. Even though a BSC has a face shield, eye protection must be worn in the laboratory. Safety glasses with side-splash protection are recommended; even if the worker wears corrective eyeglasses safety glasses should be worn over them. Goggles or a full-coverage face shield may substitute for safety glasses, and must be used if procedures that could generate splashes have to be performed outside the BSC.

V. Use of Infected or Exposed Animals

1) Containment Practices. When animals are exposed or infected with adenoviruses or constructs utilizing adenovirus vectors, ABSL-2 facility and practices must be used. The Institutional Biological and Recombinant DNA Safety Committee and the Institutional Animal Care and Use Committee must both approve the work, and a DLAM biohazard warning sign must be filled out and posted on the animal room door.

2) Shedding. Infected animals may shed adenovirus, especially during the first 72 hours after exposure. Precautions must be taken not to create aerosols when emptying animal waste material and when washing cages or cleaning animal rooms.

3) Necropsy. Necropsy should be performed using BSL-2 practices. Tissues collected for analysis are transported to the lab according to the requirements detailed in section III-6 above.

4) Training. It is recommended that animal care for infected animals be provided by lab personnel or animal husbandry technicians specifically trained in the hazards associated with the work and required ABSL-2 practices and procedures.

VI. Exposure Management

1) Eye exposures. If infectious or potentially infectious liquids or aerosols splash into the eyes, rinse immediately for a minimum of 15 minutes in an eyewash station, or using

a drench hose. Report the incident immediately to the principal investigator and the biosafety office.

2) Needlesticks. An exposure to non-intact skin or a parenteral needlestick should be scrubbed thoroughly with soap and warm water, then treated with a 10% povidone iodine solution (such as Betadine™). Report the incident immediately to the principal investigator and the biosafety office.

3) Symptoms. If any of the following symptoms occur in a researcher who works with adenoviruses, whether a recognized exposure has occurred or not, report immediately to the biosafety office. Treatment will be coordinated with OES and Risk Management offices.

- acute respiratory symptoms; cough, labored breathing, sneezing
- pleural pain or difficulty breathing
- corneal inflammation / scarification
- conjunctival infection (pink eye)

4) Reporting. All exposures must be reported to the laboratory principle investigator, who will document them. An Occupational Accident or Illness Report must be filled out and signed by the supervisor of the exposed worker and forwarded to LSU Risk Management within five days of the exposure (see PS-90 or go to the risk management web site at <https://www.lsu.edu/riskmgt/> for specific instructions). The Workers Compensation Coordinator can be reached at 578-3285. When an exposure occurs, the principle investigator will also contact the Biological Safety Manager at 578- 4658, who will complete an incident report. The incident report should contain as much of the following information as possible:

- a. a description of the exposed person's duties that relate to the exposure incident,
- b. the route of exposure and detailed circumstances of the exposure,
- c. the infectious, or potentially infectious material to which the employee was exposed; blood, culture fluid, etc.