PI Name:

Safety Protocol for working with Canine Adenovirus

1. Hazard communication statement

Adenoviruses commonly used in biomedical research laboratories are generally categorized as Risk Group 2 (RG2) agents, which are associated with human disease that is rarely serious and for which preventative or therapeutic interventions are often available. Biosafety level 2 containment is used for work with RG2 agents.

Adenoviruses are non-lipid enveloped viruses with DNA genomes that rarely integrate into the host genome. Common modes of transmission include inhalation, ingestion and contact mediated transmission. Adenoviral infections can result in conjunctivitis, upper respiratory infection and skin rash. Trans-placental infection can lead to birth defects. Immuno-compromised individuals and/or pregnant individuals are at increased risk. Non-lipid enveloped viruses such as Adenovirus are more resistant to disinfection, survive well outside of the laboratory environment and can be easily transmitted via contaminated clothing. Centrifugation should be performed using sealed tubes and sealed rotors or safety cups to contain aerosols. Freshly made 10% bleach should be used as a disinfectant. Weaker disinfectants like ethanol and quaternary ammonium compounds (ingredients in Pine Sol© and Cavicide©) or Phenols should not be used. Personnel infected with viral vectors can exhibit expression of insert genes. Oncogenes and immune modulator genes are of special concern.

Canine adenovirus (CAV) primarily infects canines (dogs, wolves, coyotes) as well as bears and foxes, but has not been reported to produce disease in humans (Carter and Wise, 2006). Two types of CAV have been identified. CAV-1 can cause severe infection, potentially leading to fatal hepatitis in canines. In contrast, CAV-2, which is used in this protocol, produces a relatively mild upper respiratory illness, characterized by coughing, rhinitis, and fever that typically resolves in a few days. In addition, our viral construct is E1-deleted and cannot replicate unless complemented my canine E1. The E1-deleted CAV-2 does not recover replication ability even in the presence of human cells that are capable of trans-complementing E1-deleted CAV (Kremer et al 2000). Published reports in rodents also show that infection in brain tissue of E1-deleted CAV-2 remains restricted to the neurons initially infected by the virus and does not spread further (Hnasko et al, 2006)

References:

Carter GR, Wise DJ (2006) Adenoviridae. A concise review of Veterinary Virology. http://www.libyanvet.com/concisereviewofveterinaryvirology.htm

Kremer EJ et al (2000) Canine adenovirus vectors: an alternative for adenovirus mediated gene transfer. J Virol. 74:505, PMC111562

Hnaski TS et al (2006) Cre-recombinase-mediated restoration of nigrostriatal dopamine-deficient mice reverses hypophagia and bradykinesia. PNAS 103: 8857, PMC1466546

2. Laboratory Precautions

1. Standard Laboratory practices

Viruses shall be handled with appropriate precautions consisting primarily of **good microbiological laboratory techniques** as well as Biosafety Level 2 (BSL-2) containment. The following precautions should be employed:

A. Access to the laboratory is limited or restricted at the discretion of the laboratory director.
B. Placards should be placed on the entrances to the lab listing biological hazards and the PI's name and 24/7 contact information for the PI and/or laboratory personnel familiar with the biohazard.
C. Do not store food in lab.
D. Do not eat, drink, smoke, handle contact lenses, apply cosmetics (including chap stick), etc. in the lab.

E. Do not mouth pipette.
F. Plants or animals not involved in experiments are not allowed in the lab.
G. Laboratory personnel must be appropriately trained.
H. The safety protocol (SOP) serves as training documentation and reference information. A copy signed by laboratory personnel should be stored in the lab's safety manual.
I. Vacuum lines must be HEPA filtered
J. Liquids should be handled carefully to minimize creation of splashes and aerosols. Centrifugation should be performed using sealed tubes and sealed rotors or safety cups.
K. Sharps should be handled with extreme caution to avoid cuts or autoinoculation during use and disposal. Needles should not be bent, sheared, or recapped. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, by autoclaving or incineration.
L. Transport: Infectious or biohazardous materials must be transported in a sealed primary container inside a sealed durable and leak proof secondary container that has been labeled with a biohazard sticker
M. Lab personnel must wash their hands after they handle viable materials and animals, after removing gloves, and before leaving the laboratory or animal facility.

2. Personal Protective Equipment (PPE)

A. Wear protective gear including disposable gloves and a cloth or disposable lab coat.
    - If using a cloth lab coat, it must remain in the cell culture room, virus procedure room or inside a biohazard bag in the lab.

B. Wear safety glasses and face protection when splashes, sprays or aerosols can be expected.
C. Dispose of contaminated PPE in biohazard bags/containers.
D. No personal protective equipment shall be worn outside of the lab.

3. Working Procedures for Viral Vectors

Before working with virus:

- prepare a solution of 10% bleach in water in appropriate containers for disinfecting supplies that may come in contact with the virus. This solution should at minimum be prepared fresh weekly.
- put on PPE

While working with virus:

- always use aseptic technique
- avoid the spread of contamination and immediately replace gloves, if contamination is suspected.

After working with virus:

- If applicable, vacuum lines will be rinsed with 10% bleach.
- Treat liquid waste with bleach to a final concentration of 10% bleach for a minimum contact time of 30 minutes. After 30 minutes, dispose of liquid waste in a sink with copious amounts of running water.
- Solid waste including pipettes, containers, etc. that come in contact with virus must be disinfected with 10% bleach prior to disposal in biohazard waste container. Autoclave biohazard waste upon completion. Dispose of solid wastes in in orange bags, which are autoclaved and placed in a red biohazard bag/container for final disposal.
- Following disposal of the liquid waste, rinse glassware with 10% bleach, followed by washing and autoclaving.
- Disinfect work surfaces with 10% bleach. Bleach can corrode metal surfaces, but this can be avoided by wiping the surface with water or 70% ethanol after decontaminating to remove the bleach.
- Remove disposable PPE and dispose of it in biohazard bags.
- Wash hands with soap and water.

3. Emergency procedures

3A. Spills of virus:

1) Notify workers in the area.
2) Leave the area for 15 minutes to allow aerosols to settle. Replace contaminated PPE.
3) Upon return, mix spill with freshly made bleach to 10% final concentration.

4) Allow 30 minutes of contact time for disinfection.
5) Absorb spill with paper towels and dispose them into biohazard bags.
6) Use dustpan and broom to sweep up debris. Broken glass must be deposited into broken glass or sharps box.
7) Wipe the spill area clean using 10% bleach.
8) Dispose of contaminated PPE in autoclavable biohazard bags.

3B. In the event of injury or exposure

1) **CLEANSE WOUND:** Wash all wounds immediately with antiseptic soap and a high volume of water for up to 15 minutes.
2) **CONTROL BLEEDING**
3) **ACCIDENTAL INGESTION:** Rinse mouth with water but do not swallow.
4) **SEEK IMMEDIATE MEDICAL FOLLOW-UP** (*do not wait 24 hrs)*

Employees *and* students go to:

- **Employee Health Services** (during business hours: Monday-Friday, 7:30 am -4 pm).
  Address/Location: 57 Bee Street, Charleston SC 29425; Phone: (843) 792-2991
- **MUSC Emergency Room** (after business hours)
  Address/Location: 96 Jonathan Lucas Street, Charleston SC 29425

5) **REPORT EXPOSURE IMMEDIATELY** to your supervisor or Principal Investigator and notify Biosafety Officer (843-792-3604).
6) **NOTIFY** Employee Health Services within 24 hours by filing a Workplace Injury form at Care Team Member/Workplace Injury Form

By signing below I attest that I have read and understood these safety instructions and agree to adhere to these rules at all times. Furthermore, I feel I have been properly notified and trained of the hazards in this laboratory.

Name (print) Signature

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