PI Name:

Safety Protocol for working with Adeno-Associated Virus

The MUSC Institutional Biosafety Committee requires Biosafety Level 2 (BSL-2) containment and safety practices for work involving Adeno-Associated Virus (AAV).

1. Hazard Communication Statement

Adeno-Associated Virus (AAV) is composed of a non-lipid enveloped capsid containing a single stranded DNA genome. Upon infecting a human host cell, the viral genome preferentially integrates at a specific site in chromosome 19, where it can remain latent until the cell is superinfected with a helper virus (e.g. adenovirus, herpes virus, human papillomavirus or vaccinia virus). Helper virus mediated complementation allows for replication of AAV and shedding of infectious viral particles.

Recombinant AAV based vectors in which the Rep gene has been deleted have lost the ability to preferentially integrate and may randomly integrate into host genomes, form episomes or randomly recombine with host genomes. There is a theoretical risk of insertional mutagenesis, which may result in an increased cancer risk. Cells and tissues infected with viral vectors may exhibit expression of insert genes affecting their normal function. Oncogenes, immune modulators and toxins may cause increased cancer risk, inflammation and cell death, respectively.

AAV has not been identified as the causative agent of human disease. However, AAV has been associated with several conditions affecting human reproduction. The presence of AAV in testicular tissue and semen is associated with male infertility. AAV DNA has also been identified in cervical and uterine biopsies, suggesting sexual transmission. A significant correlation was found between the presence of AAV DNA in amniotic fluids and premature amniorrhexis (rupture of amnion) and premature labor. The presence of AAV in amniotic fluids also suggests the possibility of vertical transmission (from mother to fetus). Maternal infection in the first trimester as shown by IgM antibodies against AAV have been associated with increased risk for pre-eclampsia, intra-uterine growth restriction (birth weight below the 10^{th} percentile for gestational age), spontaneous preterm delivery and stillbirth. AAV infection of fertilized mouse eggs led to impaired embryonic development and infection of pregnant mice led to fetal death and spontaneous abortion. Factors that may predispose individuals to increased risk of complications from infection include immune-compromised status or pregnancy.

Modes of transmission include ingestion, inhalation of aerosols or droplets, contact with mucous membranes (including sexual contact) and indirect contact with contaminated surfaces or fomites. AAV can be shed through saliva, urine, feces, blood and sperm. The viral host range includes a wide range of mammals, including humans. AAV is infectious to humans and recombinant AAV based vectors have been utilized for clinical trials involving human gene transfer (i.e. gene therapy).

Non-lipid enveloped viruses such as AAV are resistant to low level disinfectants, survive well outside of the laboratory environment and can be easily transmitted via contaminated clothing. AAV particles are resistant to a wide pH range (pH 3-9) and can resist heating at 56^{o}C for 1 hour. AAV can remain infectious for up to a month at room temperature following simple desiccation or lyophilization. Contaminated materials must be chemically decontaminated with a suitable disinfectant (e.g. freshly prepared 10% bleach) or autoclaved. Weaker disinfectants such as 70% ethanol, quaternary ammonium compounds (ingredients in Pine Sol©, Lysol and Cavicide©) and phenols should not be used as they have limited efficacy.

Certain procedures can increase the risk of exposure to AAV. Centrifugation should be performed using sealed tubes and sealed rotors or safety cups to contain aerosols. Concentrated viral stocks may contain up to 10^{12} transducing units/mL, drastically increasing the severity of potential contamination or infection beyond what would be observed from exposure to clinical specimens. Whenever possible, manipulations of AAV should be performed in a biosafety cabinet. Special precautions should be taken to avoid exposure from accidental needle sticks, splashes or aerosols.

References

1. Arechavaleta-Velasco, F., et al. 2008. Adverse reproductive outcomes in urban women with adeno-associated virus-2 infections in early pregnancy. Human Reproduction. 23: 29-36
2.Botquin et. al, 1994. Adeno-associated virus type 2 interferes with early development of mouse embryos. *J. General Virology*, 75, 2655-2662
3.Burguete, T. et al. 1999. Evidence for infection of the human embryo with adeno-associated virus in pregnancy. *Human Reproduction*. 14:2396-401
4. Centers for Disease Control and Prevention and National Institutes of Health. 2006. *Biosafety in Microbiological and Biomedical Laboratories, ed. 5.* U.S. Department of Health and Human Services, Washington, DC
5.Erles, K. et. al. 2001. DNA of adeno-associated virus in testicular tissue and in abnormal semen samples. *Human Reproduction*. 16:2333-7
6.Rohde V. et. al. 1999. Detection of adeno-associated virus in human semen: does viral infection play a role in the pathogenesis of male infertility? *Fertility and Sterility*. 72:814-6.
7. Tenenbaum, L., E. Lehtonen and P.E. Monahan. 2003. Evaluation of risks related to the use of adeno-associated virus-based vectors. *Current Gene Therapy*. 3:545-65.
8. Walz, C.M. et. al. 1997. Interaction of human papillomavirus type 16 and adeno-associated virus type 2 co-infecting human cervical epithelium. *Journal of General Virology*. 78, 1441-1452.
9. Walz, C.M. et. al. 1998. Detection of infectious adeno-associated virus particles in human cervical biopsies. *Virology*. 247, 97-105

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

  Be prepared to discuss the nature of the virus and risks of rDNA with the physician.

5) **REPORT EXPOSURE IMMEDIATELY** to the 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	
_____	_____	Date: _____
_____	_____	Date: _____
_____	_____	Date: _____
_____	_____	Date: _____
_____	_____	Date: _____
_____	_____	Date: _____
_____	_____	Date: _____
_____	_____	Date: _____
_____	_____	Date: _____
_____	_____	Date: _____
_____	_____	Date: _____
_____	_____	Date: _____