TITLE:

EHS-415 GUIDELINES FOR WORKING WITH VIRAL VECTORS

I. Summary

This document provides essential information for researchers working with adeno-associated viral (AAV), adenoviral (AV), and lentiviral (LV) vector systems used in laboratories and animal research facilities. The use of all viral vectors requires approval by Emory University's Institutional Biosafety Committee (IBC).

II. Adeno-Associated Viral Vectors (AAV)

Adeno-associated viruses are in the Parvoviridae family of viruses and are considered non-pathogenic to humans even though AAV viral DNA can integrate into the host genome. Co-infection with other viruses, primarily adenoviruses, is required for AAV to replicate.

Appendix B-I of the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules[1] assesses AAV and recombinant AAV which lacks viral DNA as Risk Group 1 (RG1) agents. RG1 agents are not associated with disease in healthy adult humans. Examples of RG1 agents include all serotypes of adeno-associated virus (AAV) and recombinant or synthetic AAV constructs in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the **absence of a helper virus**.

A. Biosafety Level (BSL) Requirements when working with AAV: To ensure the ongoing protection of research staff with the increasing use of viral vectors, the following guidelines have been adopted for work with AAV vectors.

1. **Animal Biosafety Level 1 (ABSL1) Requirements** may be used for AAV work **only if the following three requirements are met**:
   1) The transgene does not express an oncogenic protein or toxin.
   2) The AAV is generated without using adenovirus or any other helper virus of human origin.
   3) The AAV is propagated in insect cell lines or purified.

2. **BSL2 and ABSL2 Requirements (described below)** must be used when any of the following apply:
   - The transgene(s) express an oncogenic protein or toxin.
   - A helper virus of human origin is used to generate AAV.
   - AAV is propagated in human cell lines without further purification before use.

B. Emerging Safety Concerns: AAV has been associated with potentially dose-limiting-toxicities in animal models and in clinical trials[2,3]. If using a needle to administer any viral vector, including AAV, consider using needle-safe devices and restraining animals using chemical or mechanical methods.

III. Adenoviral (AV) and Lentiviral Vectors (LV)

A. Viral Vector Requirements:
Adenoviral vectors and lentiviral vectors that are replication incompetent can still infect and transduce cells, often in a wide range of host cells. Emory EHSO's Biological Agent Reference Sheet (BARS) provide important safety

information about these viral vectors[4,5]. If possible, replication incompetent lentiviral vectors should be used. 3rd generation systems and above are preferred because components necessary for virus production are split across additional plasmids. If possible adenoviral vectors that are used should also be replication incompetent. Regardless of the system used, experiments using adenoviral or lentiviral vectors should be conducted at BSL2 conditions meeting the requirements described below.

B. BSL2 Requirements:

Lentiviral and adenoviral vectors should be used in laboratories at BSL2 conditions that include:

1) Containment level 2 facilities, equipment, and operational practices
2) No open-bench work should be performed.
3) All work, including administering adenoviral and lentiviral vectors to animals, should be performed inside a Class II biosafety cabinet (BSC).
4) The use of needle-safe sharps is encouraged.
5) Centrifuge rotors must have a sealed lid, samples should be loaded/unloaded inside the BSC, and the centrifuge should be decontaminated with manufacturer recommended disinfectant after use.

C. ABSL2 & ABSL1 Requirements and Procedure:

Following viral administration, animals can still have infectious virus on their wound or in body secretions. To reduce the potential of viral transmission to research staff, it is important that the step-down procedure used to move animals from ABSL2 to ABSL1 be carefully followed. Unless otherwise specifically approved by the Institutional Biosafety Committee (IBC) of Emory University, the following steps should be followed when using lentiviral and adenoviral vectors for gene delivery in animal models. These guidelines are based on The National Institutes of Health (NIH) Office of Science Policy (OSP) Biosafety Considerations for Research with Lentiviral Vectors[6].

Step 1: **Only replication-incompetent lentiviral vectors produced with established 3 and 4-plasmid systems (or higher) or adenoviral vectors produced without helper virus that pose minimal risk of replication-competent virus should be selected for the purpose of gene transfer into animals.**

Step 2: The initial delivery of viral vector should be performed under BSL2 conditions in a biosafety cabinet with the animals subsequently housed in ABSL2 conditions in cages with filter tops placed in a designated containment area. If using a needle to administer viral vector, consider using needle-safe devices and restraining animals using chemical or mechanical methods.

Step 3: If recipient animals are approved by IACUC for transfer from the laboratory to the animal research facility (ABSL2), the animals must be transported in **filter top cages**.

Step 4: Once the period of potential infectivity is over, the containment level can be reduced to ABSL1 following the procedures below:

a) Following infection with lentivirus or adenoviral vectors, animals are housed in ABSL2 vivarium space for at least 72 hours following DAR guidelines.
b) Cage signage should follow DAR guidelines.
c) 72 hours following infection, animals can be transferred to ABSL1 standard conditions following DAR guidelines.
d) Once animals have been transferred to ABSL1 (stepped down), they can then be used within ABSL1 behavioral facilities, etc., as with other ABSL1 animals. After being stepped down, long-term use of rodents infected with replication incompetent viral vectors within animal biosafety 1 (ABSL1) conditions is generally considered acceptable.

IV. Viral Vectors

If you will be working with viral vectors (e.g., AAV vectors, AV vectors, LV vectors, retroviral vectors, poxviral vectors, herpes viral vectors, rabies viral vectors, alpha viral vectors, baculoviral vectors, etc.), please complete a Viral Vector Registration Form in SciShield (previously BioRAFT) and provide a description of the planned research in the respective Biological Research Project Form in SciShield. Include sufficient detail (e.g., any pseudotyping, description of production methods, description of safety features, etc.) to allow the IBC to review the proposed use of the viral vector. If the LV vector stock is thawed and a dilute virus particle suspension is prepared for use inside a biosafety cabinet (BSC) before transferring a small volume of the dilution to stereotaxic equipment, this should be indicated in the viral vector form.

V. References

1. NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules. April 2024. Department of Health and Human Services. National Institutes of Health.
2. Flotte TR. Revisiting the “New” Inflammatory Toxicities of Adeno-associated Virus Vectors. Hum Gene Ther 2020; 31:398-399.
3. Hinderer C, Katz N, Buza EL et al. Severe Toxicity in Nonhuman Primates and Piglets Following High-Dose Intravenous Administration of an Adeno-Associated Virus Vector Expressing Human SMN. Hum Gene Ther 2018; 29:285-298.
4. Emory EHSO, Biological Agents Reference Sheet (BARS), Adenovirus and Adenoviral Vectors, https://ehso.emory.edu/resources/bars/adenovirus.html
5. Emory EHSO, Biological Agents Reference Sheet (BARS), Lentivirus and Lentiviral Vectors, https://ehso.emory.edu/resources/bars/lentivirus.html
6. The National Institutes of Health (NIH) Office of Science Policy (OSP) https://osp.od.nih.gov/wp-content/uploads/Lenti_Containment_Guidance.pdf