Institutional Biosafety Committee - UTEP	
Title: **IBC Standard Operating Procedure – Adeno-Associated Virus Vectors**	
Date in Effect: 8 October 2025	**Revision Date:**
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A. Background

Adeno-associated virus (AAV) and recombinant adeno-associated virus (rAAV) are commonly used as vectors for gene delivery due to their high safety profile, non-enveloped structure, and minimal immunogenicity. The rAAV vectors are replication-defective and do not integrate efficiently into the host genome, thereby posing fewer long-term biosafety risks.

Recombinant AAV is engineered to carry only the gene of interest flanked by inverted terminal repeats (ITRs) and lacks viral coding sequences. These vectors are dependent on co-transfection with helper plasmids encoding rep and cap genes, along with adenoviral helper functions, to be packaged in producer cells. rAAV is then purified and used to transduce target cells.

B. AAV Vector Biosafety Characteristics

Adeno-associated virus (AAV) vectors are widely considered among the safest viral vectors for laboratory use. They are non-enveloped, replication-defective, and typically remain episomal within host cells, which minimizes the risk of insertional mutagenesis. Although the NIH classifies AAV as a Risk Group 1 agent, biosafety precautions are still necessary due to the potential for immune responses, off-target effects from the transgene, and systemic exposure. These concerns are especially important when working with high vector titers or when the genetic cargo poses additional hazards.

In accordance with the OSHA Bloodborne Pathogens (BBP) Standard (29 CFR 1910.1030), research institutions must minimize personnel exposure to human-derived infectious materials, including recombinant nucleic acid molecules. At many institutions, including those following NIH and CDC guidelines, this includes vectors produced using human cells or components.

The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines) identify AAV types 1-4, and rAAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product (for example, an oncogene) or a toxin molecule, and are produced in the absence of a helper virus as risk group 1 (RG1) agents, which are not associated with disease in healthy adult humans (see Appendix B-1 of the NIH Guidelines).

While some AAV vectors grown in insect cells without human-derived helper viruses may qualify for BSL/ABSL-1 containment, most AAV vectors are produced in human cell lines such as HEK293. Procedures using human cell lines under the BBP standard and must be handled using BSL/ABSL-2 practices, unless stringent purification and quality control (QC) procedures are applied.

[1]MWRI, Addgene, and Vector Biolabs are recognized core production facilities with purification and quality assurance procedures for AAV/rAAV produced from human cells that may be used at BSL/ABSL-1.

The Institutional Biosafety Committee (IBC) requires:

- Purification of AAV vectors produced in human cells to remove potential human pathogens.
- Verification of purification using SDS-PAGE, silver staining, or similar QC methods.

This purification ensures not only biosafety but also improves experimental reproducibility and reliability—factors critical for publication and grant submission.

The IBC may approve the use of AAV/rAAV at BSL/ABSL-1 only if all the following criteria are met[1]:

- The transgene does not encode an oncogene or known toxin.
- AAV/rAAV is generated without a helper virus of human origin (including helper plasmids).
- AAV/rAAV is propagated in non-human/insect cell lines.

AAV or rAAV must be used at BSL/ABSL-2 if:

- It is propagated in human-derived cell lines (e.g., HEK293) without further purification before use.
- It includes helper functions or plasmids of human viral origin.
- Transgenes express an oncogenic protein or toxin

C. Health Hazards and Risks of AAV

Adeno-associated virus (AAV) vectors are generally considered low-risk biological agents due to their non-pathogenic nature and replication-incompetence in the absence of a helper virus. However, potential risks arise from high-titer preparations, immunogenic responses to capsid proteins, and the nature of the delivered transgene.

Potential Health Effects:

AAV is not associated with any human disease; however, there is evidence of AAV infection in the human embryo and an association of AAV with male infertility.

Post-Exposure Concerns:

Supportive care. No specific Treatment/Prophylaxis

Personnel Exposed to Biohazard

If a BSL/ABSL-2, exposure occurs it must be reported to the Principal Investigator and EH&S. Contact the Biosafety Officer if unsure of proper response and complete the EH&S Incident and Injury report form found at https://www.utep.edu/ehs/_files/docs/forms/ehs-injury-incident-report.pdf.

D. Biosafety Precautions

Proper biosafety practices are critical when handling BSL/ABSL-2 AAV vectors due to the risk of exposure to laboratory personnel. The following biosafety precautions must be always followed to ensure containment and personnel safety.

[1]MWRI, Addgene, and Vector Biolabs are recognized core production facilities with purification and quality assurance procedures for AAV/rAAV produced from human cells that may be used at BSL/ABSL-1.

- Perform all manipulations within a certified biosafety cabinet (BSC).
- Wear appropriate PPE: gloves, lab coat, etc.
- Follow BSL-2 containment practices if vector includes human-derived materials or hazardous transgenes.

Engineering Controls:
Biosafety cabinet (Class II) is required for all manipulations for BSL/ABSL-2 AAV. Use sealed centrifuge cups when using a centrifuge and safety-engineered sharps when necessary or avoid where possible for BSL/ABSL-2 AAV.

Animal Work:
Animals inoculated with AAV vectors are housed at ABSL-1 or ABSL-2 depending on the transgene. Soiled bedding and cages are treated as biohazardous for at least 72 hours post-injection for BSL/ABSL-2 AAV only. Inoculations and cage changes are performed in a biosafety cabinet for BSL-2 AAV only.

Spill Response:
Evacuate and allow aerosols to settle for 30 minutes. Wear proper personal protective equipment (PPE) when entering the room, then cover spill with paper towels and apply disinfectants. Allow 30 minutes contact before cleaning and disposal. For large spills, contact Environmental Health & Safety Spill Response Team.

E. Decontamination and Waste Disposal

Proper decontamination and disposal procedures are essential to minimize the risk of environmental contamination and occupational exposure. For surfaces, disinfect work surfaces and equipment with 1:10 bleach (minimum contact time: 30 seconds) or 70% ethanol. Ensure thorough coverage from the outer edge of the spill toward the center. BSL-2 AAV vectors can be inactivated by heating at 56°C for at least 30 minutes**.**

Waste Disposal:
All disposable materials (pipette tips, gloves, wipes) in contact with AAV must be discarded into biohazard waste containers. Liquid waste containing AAV must be disinfected with bleach (1:10 dilution) for a minimum contact time of 30 minutes to ensure inactivation before disposal. Animal bedding and waste from inoculated animals must be handled as regulated medical waste (RMW) for at least 72 hours post-injection.

F. References

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH, 2023) - https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf

Cornell University EHS. (2024). *Adeno-Associated Virus (AAV) Biosafety Guidelines*.

NIH. (2023). *Biosafety Considerations for Research with Recombinant AAV Vectors*. Office of Science Policy.

CDC. (2020). *Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th Edition*. https://www.cdc.gov/labs/bmbl/

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Grimm, D. & Kay, M.A. (2003). *From virus evolution to vector revolution: use of naturally occurring serotypes of AAV for gene therapy*. *Hum Gene Ther*, 14(11): 1045–1056.

ASGCT. (2021). *Safety of AAV Gene Therapy Vectors*. American Society of Gene & Cell Therapy.

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