

Institutional Biosafety Committee (IBC) Guidance on Biosafety Level Assignment for Adeno-Associated Virus (AAV)

Background:

Adeno-associated virus (AAV) and recombinant adeno-associated virus (rAAV) are commonly used for gene expression with fewer associated biosafety concerns when compared to viral vectors that are persistent and able to integrate into the genome. Historically, the IBC has assigned all work with AAV/rAAV to Biosafety Level 2 (BSL-2) or Animal Biosafety Level 2 (ABSL-2). The following is guidance for determining the appropriate biosafety designation when working with AAV/rAAV vectors.

NIH Opinion:

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 adults humans (*NIH Guidelines Appendix B-1*).

IBC Guidance:

The VAPHS IBC will utilize the following criteria for determining appropriate biosafety containment and handling of AAV/rAAV:

- Propagation with or without helper virus, including the use of adenovirus
- Presence of transgenes encoding oncogenes or toxins
- Propagation in insect cell lines versus human cell lines
- Assurance of purification techniques and quality control methods used when propagation of virus occurs in human cell lines

Specific Requirements For Use of AAV/rAAV Use at BSL-1/ABSL-1

The IBC will consider designating AAV or rAAV for use at BSL-1/ABSL-1 if the following three criteria are met:

1. Transgene does not express an oncogenic protein or toxin (*NIH Guidelines reference Section III-B-1*)
2. AAV/rAAV is generated without using adenovirus or any other helper virus of human origin
3. AAV/rAAV is propagated in insect cell lines

Determination of the biosafety level for AAV/rAAV meeting conditions 1 and 2 above and propagated in human cell lines will be made by the IBC on a case-by-case basis for each vector lot, when specific requirements have been addressed (see below "*Exceptions to the requirement for BSL-2/ABSL-2*").

Specific Requirements For Use of AAV/rAAV Use at BSL-2/ABSL-2

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

1. Transgenes express an oncogenic protein or toxin
2. Helper virus of human origin is used to generate AAV/rAAV
3. AAV/rAAV is propagated in human cell lines ***without*** further purification before use

***Exceptions to the Requirement for BSL-2/ABSL-2**

AAV and rAAV are typically propagated in human embryonic kidney (HEK) 293 cells, a commercially available human cell line. Under the Code of Federal Regulations 29 CFR 1910.1030 (otherwise known as the Bloodborne Pathogens Standard), all human-derived materials are to be handled under BSL-2 conditions (Universal Precautions), per the Center for Disease Control (CDC) and the Occupational Safety and Health Act (OSHA) regulations.

The IBC will consider reducing the biosafety level to BSL1/ABSL-1 on a *case-by-case basis* when the following criteria are met and documented in the protocol application. These requirements are in addition to the oncogene/toxin expression and helper virus criteria listed above:

- A. AAV/rAAV generated in non-human cells or AAV/rAAV generated in human cells by a helper virus-free plasmid transfection method with subsequent purification and appropriate quality control.

The investigator must provide details of the methodology for purification and quality control on the protocol application, for example:

- a. purification by cesium chloride or iodixanol gradient, and/or column chromatography followed by
- b. quality control using SDS/PAGE gel electrophoresis

*All investigators who receive IBC approval for specified rAAV at a downgraded biosafety containment level (BSL-1/ABSL-1) are required to keep the AAV quality control data for the specific vector source in the laboratory records.

Similar purification and quality control methods may be used to justify application for work with AAV/rAAV obtained from other sources.

- B. AAV viruses acquired from a recognized core facility should include the method used for generating, purification, and quality control methodology from the core facility.
- C. Investigators who are not generating their own viruses but are acquiring viruses from another laboratory to which an IBC has granted approval to use AAV/rAAV at BSL-1/ABSL-1, should provide the name of the institution, the approved protocol number and the name of the investigator providing the AAV/rAAV. In this case, a detailed description of the method used for generating, purification, and quality control methodology may be omitted from the application.

Table 1: Summary of biosafety level requirements for AAV/rAAV use

Oncogene or Toxin?	Helper Virus of Human Origin (e.g., human adenoviruses and herpes virus)?	Propagated in Human Cell Line (e.g., HEK 293 cells)?	Biosafety/Animal Biosafety Level
Yes	Yes	Yes	With purification and QC: BSL-2/ABSL-2 Without purification and QC: BSL-2/ABSL-2
		No	BSL-2/ABSL-2
Yes	No	Yes	With purification and QC: BSL-2/ABSL-2 Without purification and QC: BSL-2/ABSL-2
		No	BSL-2/ABSL-2
No	Yes	Yes	With purification and QC: BSL-2/ABSL-2 Without purification and QC: BSL-2/ABSL-2
		No	BSL-2/ABSL-2
No	No	Yes	With purification and QC: BSL-1/ABSL-1 Without purification and QC: BSL-2/ABSL-2
		No	BSL-1/ABSL-1