The University of Texas at San Antonio
Institutional Biosafety Committee
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. The following is a brief synopsis of IBC guidance relevant to biosafety with respect to AAV/rAAV vectors.

Historically, the IBC has assigned all work with AAV/rAAV to Biosafety Level 2 or Animal Biosafety Level 2 (BSL/ABSL-2). The IBC, at its convened meeting on 6 November 2013, voted to adopt this Policy, by which AAV/rAAV could be safely handled at Biosafety Level 1 or Animal Biosafety Level 1 (BSL/ABSL-1). The following guidance was adopted by the UTSA IBC on 6 November 2013.

NIH opinion

The NIH Guidelines for Research Involving Recombinant DNA 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).

IBC Guidance

The University of Texas at San Antonio 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
- Purification techniques and quality control methods used when propagation of virus occurs in human cell lines

<u>Specific requirements of AAV/rAAV use at BSL/ABSL-1</u>

The IBC will consider designating adeno-associated viruses or recombinant adeno-associated viruses for use at BSL/ABSL-1 if:

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 when specific requirements have been met (see below “Exceptions to the requirement for BSL/ABSL-2”)

Specific requirements of AAV/rAAV use at BSL/ABSL-2

Adeno-associated viruses or recombinant adeno-associated viruses must be used at BSL/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/ABSL-2

AAV/rAAV are typically propagated in HEK 293 cells, a commercially available human cell line. Under the Code of Federal Regulations (29 CFR 1910-1030) otherwise known as the Blood Borne Pathogen Standard, all human-derived materials are to be handled under BSL-2 (Universal Precautions) conditions, per CDC and OSHA regulations.

The IBC will consider reducing the biosafety level to BSL/ABSL-1 on a case-by-case basis for investigators who are generating AAV/rAAV in their own laboratories when the following criteria are met and documented in the IBC protocol application in addition to the oncogene/toxin expression and helper virus criteria listed above:

- 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 IBC application
    - For example, purification by cesium chloride or iodixanol gradient, and/or column chromatography followed by quality control using SDS/PAGE gel electrophoresis or similar purification and quality control methods may be used to justify application for work with AAV/rAAV at BSL/ABSL-1
- Investigators who are not generating their own viruses but are acquiring viruses from a recognized core facility should provide the method used for generating, purification, and quality control methodology from the core facility. A 'Certificate of Analysis', documenting that the core facility has analyzed the virus/vector as indicated above, should be maintained by the PI for the duration of the IBC protocol, or a minimum of 3 years.
- Investigators who are not generating their own viruses but are acquiring viruses from another UTSA laboratory to which the IBC has granted approval to use AAV/rAAV at BSL/ABSL1, should provide the IBC registration number(s) and the name of the UTSA investigator(s) providing AAV/rAAV. In this case, 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	Helper Virus of human origin (e.g., human adenoviruses and herpesviruses)	Propagated in Human Cell Line		Biosafety/Animal Biosafety Level
Yes	Yes	Yes	Purification and QC	
			Yes	BSL/ABSL-2
			No	BSL/ABSL-2
		No		BSL/ABSL-2
	No	Yes	Purification and QC	
			Yes	BSL/ABSL-2
			No	BSL/ABSL-2
		No		BSL/ABSL-2
No	Yes	Yes	Purification and QC	
			Yes	BSL/ABSL-2
			No	BSL/ABSL-2
		No		BSL/ABSL-2
	No	Yes	Purification and QC	
			Yes	BSL/ABSL-1
			No	BSL/ABSL-2
		No		BSL/ABSL-1