Adeno-Associated Viral Vector Containment Considerations

Background: Adeno-associated viruses (AAV), family *Parvoviridae*, are non-enveloped single-stranded DNA viruses approximately 25nm in diameter. AAV can only replicate in the presence of a helper virus, typically adenovirus, herpesvirus, human papillomavirus, or vaccinia, and it transduces both dividing and non-dividing cells. In the absence of a helper virus, AAV can stably integrate into the host cell genome. A lytic cycle can be triggered by co-infection with a helper virus. Wild-type AAV preferentially integrates into human chromosome 19; recombinant vectors (rAAV) lose this specificity and appear to integrate randomly. Random integration of rAAV poses a theoretical risk of insertional mutagenesis.

AAV vector characteristics include:

- Limited cloning capacity (~4.5kb)
- Ability to be produced in high titers
- Broad host cell range

Potential Health Hazards: AAV is generally considered non-pathogenic whereby infection does not cause direct disease in humans. The presence of AAV integrated into male testis tissue and semen DNA has been reported and suggested to be associated with male infertility. Insertional mutagenesis presents a risk for tumor formation and cancer. There is no specific treatment for infection with AAV.

Transmission:

- Inhalation of aerosolized droplets
- Mucous membrane contact
- Parenteral injection
- Ingestion

Environmental Stability: AAV is stable at temperatures ranging from 4-56˚C and at pH 5.5-8.5. AAV has been shown to remain infectious for at least one month at room temperature after desiccation.

Methods of Inactivation:

- 0.5% Sodium hypochlorite for 5 minutes
- 0.25% peracetic acid for 5 minutes
- Iodine for 5 minutes
- Autoclave: 121˚C for 30 minutes

Note: AAV is resistant to alcohols, Cavicide, Gladiator, and ethylene oxide.

Risk Group: 1

The NIH Guidelines identify all AAV serotypes and recombinant or synthetic AAV constructs as risk group 1 (RG1) agents so long as the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and is produced in the absence of a helper virus.

Biosafety Containment:

The following criteria should be considered for determining the appropriate biosafety containment and handling of AAV/rAAV:

- Propagation with or without helper virus

Original IBC Review: 1/15/2020
Last Update: 1/16/20

- Presence of potentially hazardous genes (oncogenes, toxins, allergens, cytokines, siRNA to a tumor suppressor etc)
- Presence of Risk Group 2 or 3 materials
- Propagation cell line
- Verification of purification techniques and quality control assays when propagation occurs in human cell lines

Summary of biosafety level containment for AAV/rAAV use				
Contains potentially hazardous gene	Helper Virus is used	Propagation in Human Cell Lines (e.g. HEK 293 cells)	Purification and Quality Control	Recommended BSL/ABSL*
Yes	Yes	Yes	Yes	2
			No	2
		No	Yes	2
			No	2
	No	Yes	Yes	2
			No	2
		No	Yes	2
			No	2
No	Yes	Yes	Yes	2
			No	2
		No	Yes	2
			No	2
	No	Yes	Yes	1*
			No	2
		No	Yes	1*
			No	1*

*Infection of animals should occur in a BSC when possible. Due to potential vector shedding, cages should then be sealed and marked as biohazardous for the first 72 hours post infection. If a cage must be opened in the first 72 hours, it must be done in a BSC. At 72 hours, the cage bedding should be discarded as BSL2 waste. Subsequent cage changes and handling may be considered under ABSL-1 standard procedures.

References:

Farraha M., Barry M., Lu J., et al. (2019) Analysis of recombinant adeno-associated viral vector shedding in sheep following intracoronary delivery. *Gene Ther* 26(9): 399-406.

Ferla R., Alliegro M., Marteau J., et al. (2017) Non-clinical Safety and Efficacy of an AAV2/8 Vector Administered Intravenously for Treatment of Mucopolysaccharidosis Type VI. *Mol Ther Methods Clin Dev* 6: 143-158.

Howard D., Harvey B. (2017) Assaying the Stability and Inactivation of AAV Serotype 1 Vectors. *Hum Gene Ther Methods* 28(1): 39-48.

Kimura T., Ferran B., Tsukahara Y., et al. (2019) Production of adeno-associated virus vectors for *in vitro* and *in vivo* applications. *Nat Sci Rep* 9: 13601.

Original IBC Review: 1/15/2020
Last Update: 1/16/20