University of Virginia Institutional Biosafety Committee Policy for Biosafety Level Assignment of Adeno-Associated Viral Vectors (AAV)

Policy Statement: The UVa Institutional Biosafety Committee requires Biosafety Level 2 (BSL2) and Animal Biosafety Level 2 (ABSL2) containment and safety practices for experiments involving Adeno-Associated Virus (AAV).

Background: Adeno-associated viruses are members of the parvovirus family. They are non-enveloped, single-stranded DNA viruses that can only replicate in the presence of a helper virus such as adenovirus (Ad), herpes simplex virus, human papillomavirus or vaccinia for productive infection. Recombinant AAV-vectors of various serotypes which differ in their cell tropism are widely used for gene delivery in biomedical research as their characteristics include: the ability to infect a broad range of dividing and non-dividing cells; ability to be produced in high titers; long-term, stable expression and different serotypes specifically bind to different target cell populations.

Rationale: *The NIH Guidelines for Research Involving Recombinant or Synthetic DNA Molecules* (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 (e.g. oncogene) or a toxin molecule, and is produced in the absence of a helper virus. However, the following factors have contributed to the IBC assigning BSL2 containment for AAV experiments:

Potential AAV Health Hazards: While AAV is generally considered non-pathogenic; there are several reports describing AAV infection in association with adverse reproductive outcomes, including spontaneous abortion and spontaneous preterm birth (Tobiasch et al, 1994; Burguete et al., 1999; Arechavaleta-Velasco et al., 2008; Chung et al., 2012). In males, integrated AAV DNA has been detected in testis tissue samples (Mehrle et al., 2004) and the presence of AAV DNA in semen samples has been reported and suggested to be associated with male infertility (Chung et al., 2012). Further, while wild type AAV integrates preferentially into human chromosome 19 and remains latent until a helper virus supplies the necessary genes for replication, recombinant AAV vectors lose site specific integration, thereby raising the theoretical concern of insertional mutagenesis. While 90% of humans are seropositive for AAV, genomic analysis of tissues demonstrated AAV sequences are found in a variety of tissues with a hybrid of two seroforms of AAV commonly found integrated in human liver and bone marrow (Gao et all., 2004). Recent findings also provide evidence that chromosomal insertions of AAV serotype 2 increases the expression of proto-oncogenes in human hepatocellular carcinoma (Nault, et al., 2015).

- Residual Human Cell Contaminants: AAV vectors are typically propagated in HEK 293 cells either in the presence of a helper virus or by co-transfection of helper plasmids. HEK 293 are a commercially available human embryonic kidney continuous cell line. *The UVa IBC Policy on the Use of Human Cell Lines for Laboratory Personnel* requires that all cell and organ cultures of human origin, including well established cell lines be handled in accordance with the OSHA Bloodborne Pathogens Standard under BSL2 containment. Unless viral purification and quality control measures for AAV are

effectively applied, it is likely that any contaminants that may have been present in the original human cell culture will also be contained in the resulting supernatant.

Appeals: The IBC will consider requests for designating AAV experiments at BSL/ABSL-1 containment if the following information is provided:

1) The nature of transgene expression;
2) Whether or not the vector is generated using adenovirus or any other helper virus of human origin;
3) Identification of the cell line in which the vector is propagated.
4) A description of purification procedures (e.g. column chromatography, etc.) and/or documentation from the source vector core facility that describes purification procedures.
5) A description of any AAV vectors used in the laboratory which are handled at BSL2 containment.

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References

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