



BIOHAZARDOUS AGENT REFERENCE DOCUMENT

Adeno-Associated Viral Vectors (AAV)

The Biohazardous Agent Reference Document (BARD) is a general guidance resource that reviews and summarizes the nature of a pathogen or biotoxin, and offers safety requirements for work with the agent in the laboratory. The BARD may replace the formal SOPs used in conjunction with some IBC registrations.

The BARD is provided as an additional guidance tool, and is not a substitute for a risk assessment, biosafety training, lab-specific training, or a formal [IBC master protocol registration](#). This document should be readily available in the laboratory, and it is the responsibility of the Laboratory Supervisor or Principal Investigator to ensure that all personnel have read, understood, and signed the document. The BARD is for informational purposes only, and is not intended to be a substitute for professional medical advice, diagnosis, or treatment. Please consult a health care provider for any medical questions or concerns.

INSTRUCTIONS

- 1. Review the information contained in this document.**
- 2. Add any necessary information that is specific to your work in the laboratory (such as strain-specific information). Please be sure that the track changes function is turned on to indicate any changes that you make.**
- 3. Instruct all personnel to review the BARD and sign the last page, indicating that they have read and understood the information.**
- 4. Submit the BARD along with your IBC master protocol registration, amendment, or continuing review.**

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CHARACTERISTICS	
Morphology	Non-enveloped, single-strand DNA viruses that can only replicate in the presence of a helper virus (such as Adenovirus, Herpes virus, or Vaccinia). In the absence of helper virus, wild-type AAV can stably integrate into the host genome (to the AAVS1 region of human chromosome 19) and remain latent until exposed to a helper virus. Recombinant AAV loses this specificity, but may integrate randomly at a lower rate. AAV has the ability to infect a broad range of cells. Eleven serotypes have been identified.
Strain Specific Characteristics	The biosafety level of specific AAV will be evaluated on a case-by-case basis, with consideration given to: <ol style="list-style-type: none"> 1. Nature of the transgene 2. Presence of helper virus 3. Type of cell line used for propagation 4. Verification of purification when propagated in human cell lines or when helper virus is used

HEALTH HAZARDS	
Host Range	Humans and some other primates are natural hosts. Other vertebrate animals may be experimentally infected.
Modes of Transmission	Inhalation of aerosols, droplet exposure to mucous membranes, ingestion, and injection.
Signs and Symptoms	No known disease association for wild-type virus, although infection may elicit a mild immune response. Recombinant virus may integrate randomly, posing a theoretical risk of insertional mutagenesis.
Infectious Dose	Unknown
Incubation Period	Unknown

MEDICAL PRECAUTIONS / TREATMENT	
Prophylaxis	None available
Vaccines	None available
Treatment	No specific treatment
Surveillance	Monitor for symptoms
UVM IBC Requirements	Report any exposures or signs and symptoms to your supervisor
Additional Medical Precautions	

LABORATORY HAZARDS	
Laboratory Acquired Infections	None reported. Commonly used as a gene therapy delivery system.
Sources	Respiratory droplets, laboratory cultures

BSL - 1	Transgene <i>does not</i> express an oncogene or toxin, viruses generated <i>without</i> helper virus, acceptable verification that helper virus is not present, or propagation in insect cell lines
BSL - 2	Transgene that expresses an oncogene or toxin, viruses that are propagated in human cell lines <i>without</i> further purification before use, known presence of helper virus, or lack of acceptable verification of purification
ABSL - 1	Animals may be housed at ABSL-1 72 hours after administration, once the initial cage change has been completed.
ABSL - 2	Injection of animals, oropharyngeal or nasal inoculation of virus that requires BSL-2 containment. Animal bedding should be considered biohazardous for 72 hours after exposure. Filter-top cages, hazard ID cage cards, and ABSL-2 door signage must be used.
Aerosol generating activities	Centrifugation, homogenizing, vortexing or stirring, changing of animal cages, animal surgeries, cell sorting, pipetting, pouring liquids, sonicating, loading syringes
Primary containment device (BSC)	Use for all BSL-2 virus work, virus propagation, large volumes, or aerosol-generating activities

EXPOSURE PROCEDURES	
Mucous membranes	Flush eyes, mouth or nose for 15 minutes at eyewash station.
Other exposures	Wash area with soap and water for 15 minutes
Medical Follow-Up	Contact UVMCMC Infectious Disease Dept. directly at (802) 847-2700 for immediate assistance. Bring this document with you if seeking medical care.
Reporting	Report all exposures or near misses to: <ol style="list-style-type: none"> 1. Your immediate Supervisor 2. The UVM Biosafety Officer at (802) 777-9471 and Risk Management at 6-3242 3. Risk Management and Safety; http://www.uvm.edu/safety/lab/incident-reporting

PERSONAL PROTECTIVE EQUIPMENT (PPE)	
Minimum PPE Requirements	Nitrile gloves, lab coat or gown, appropriate eye/face protection. Wash hands after removing gloves.
Additional Precautions (Risk assessment dependent)	Open wounds, cuts, and scratches should be covered with waterproof dressings.

CONTAINMENT REQUIREMENTS

Principal Investigator: _____

IBC Registration #: _____

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VIABILITY	
Disinfection	Susceptible to 10% bleach, 2% glutaraldehyde, 1% iodine, or 5 % peracetic acid; with 10-minute contact time.
Inactivation	Autoclaving for 30 minutes at 121°C
Survival Outside Host	Can survive on surfaces for several weeks

SPILL CLEAN UP PROCEDURES	
Small Spill	Notify others working in the lab. Allow aerosols to settle. Don appropriate PPE. Cover area of the spill with paper towels and apply approved disinfectant, working from the perimeter towards the center. Allow 30 minutes of contact time before clean up and disposal. Dispose in double biowaste bags and biobox.
Large Spill	<p>Inside of a lab: Call UVM Service Operations at 656-2560 and press option 1 to speak to a dispatcher. Ask them to page Risk Management and Safety.</p> <p>Outside of a lab: Pull the nearest fire alarm and evacuate the building. Wait out front of the building for emergency responders to arrive.</p>

Addgene AAV Guide	https://www.addgene.org/guides/aav/
BMBL	https://www.cdc.gov/biosafety/publications/bmbl/5/
Annual Review of Genetics	https://www.annualreviews.org/doi/full/10.1146/annurev.genet.37.110801.143717?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub%3Dpubmed
UVM AAV fact sheet	https://www.uvm.edu/sites/default/files/UVM-Risk-Management-and-Safety/aav_vectors_fact_sheet.pdf
Human Gene Therapy Methods	https://www.ncbi.nlm.nih.gov/pubmed/28192678

STUDENT / EMPLOYEE NAME	SIGNATURE	DATE

Biosafety Review:

_____ Date

_____ Jeff LaBossiere, Biological Safety Officer

REFERENCES

Principal Investigator: _____ IBC Registration #: _____

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RECOGNIZED AAV PRODUCTION CORE FACILITIES		
Core	Purification Procedure	BSL
<u>UNC</u>	Iodoxinal Gradient + Column Purification QC analysis by SDS-PAGE/Silver Stain per vector per lot. Will provide purity and titer per lot.	BSL-1
<u>MWRI</u>	Iodoxinal Gradient + Column Purification QC analysis by SDS-PAGE/Silver Stain per vector per lot. Will provide purity and titer per lot.	BSL-1
<u>Addgene</u>	Iodoxinal Gradient followed by concentration QC analysis by qPCR titer, SDS-PAGE/Silver Stain. Will provide results of QC upon request.	BSL-1
<u>Salk Institute</u> (CA)	Purification on a discontinuous Optiprep™ gradient; price per prep. Custom rAAV preps are titrated using qPCR to give titer in genome copies (GC) per ml.	BSL-2 unless purification and QC data provided
<u>Stanford</u>	Provides unpurified AAV unless otherwise requested. Core facility recommends use under BSL-2.	BSL-2 unless purification and QC data provided
<u>U Penn</u>	Iodoxinal Gradient + Column Purification QC analysis by SDS-PAGE is available upon request. Will provide purity and titer per lot at cost.	BSL-2; downgrade possible with QC data provided

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SUMMARY OF BIOSAFETY LEVEL REQUIREMENTS FOR AAV USE				
Oncogene or Toxin	Human origin Helper Virus is used (e.g. human adenoviruses and herpesviruses)	Propagated in Human Cell Lines (e.g. HEK 293)	**Purification and Quality Control Documentation Required	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	2	
		No	2	
		No	2	
	No	Yes	1	
		No	2	
		No	1	

****NOTE on Purification and Quality Control:** The purification assurance (a.k.a. Quality Control or Quality Assurance) step may not be offered as a standard ordering item, and may have to be specifically requested by the researcher and therefore may incur additional product fees or pricing when purchased or obtained from a commercial vendor or another University's Vector Core. See the Recognized Core Facility chart for source information.