Pathogen Safety Sheet

AGENT NAME: Adeno-Associated Viral Vectors

RISK GROUP CLASSIFICATION: 1
*See containment section for exception and details

BACKGROUND & HEALTH HAZARDS

Adeno-associated viruses (AAV) belong to the family Parvoviridae. They are non-enveloped, single-stranded DNA viruses that can only replicate in the presence of a helper virus, adenovirus (Ad), herpes virus, or vaccinia. When there is no helper virus, AAV can insert its DNA into the host chromosome stably, preferably human chromosome 19, and remain latent until a helper virus supplies the necessary genes for replication. AAV vectors characteristics include:

- A limited cloning capacity (~4.5kb).
- Ability to be produced in high titers
- Ability to infect a broad range of cells.
- Long-term (stable) expression from randomly integrated sequences or episomal sequences.
- Replication in the presence of wild-type AAV and of a helper virus.

These viruses are infectious to humans with no known disease association. **Research involving recombinant AAV vectors must be approved by the Institutional Biosafety Committee (IBC).**

MODES OF TRANSMISSION: AAV may be transmitted by aerosol, droplet exposure to the mucous membrane, ingestion and injection.

ADMINISTRATIVE CONTROLS

ACCESS to the laboratory shall be restricted; doors must remain closed during experimentation.

SIGNS AND LABELS incorporating the biohazard symbol must be placed to indicate each area where AAV are used or stored (including biosafety cabinets, incubators, refrigerators, laboratory entrance doors, etc.) The signs should include the name of the agent, emergency contact information and a biohazard sticker. ALL laboratory personnel must be advised of the hazards of the agent.

ALL laboratory personnel must be advised of potential hazards present.

ALL laboratory personnel, including Principle Investigators, must be trained in the proper handling, use and disposal prior to working with AAV.

Standard Operating Procedures (SOP's) for the planned procedures must be written and shall be present in the laboratory at all times.
Adeno-Associated Viral Vectors

PPE must be made available to employees.

A TUBERCULOCIDAL DISINFECTANT must be available and used appropriately.

ENGINEERING CONTROLS must be made available.

ALL laboratory personnel must remove lab coats, discard gloves in the proper biological waste container and wash hands thoroughly before exiting the lab.

ENGINEERING CONTROLS

BIOSAFETY CABINET All work with AAV must be performed in a certified Class II Biosafety Cabinet (BSC) that has been inspected within the last 12 months if:

- A helper virus is present,
- Transgenes express an oncogenic protein or toxin,
- AAV is propagated in human or NHP cell lines without further purification before use.

Biological Safety Level 2 practices must be followed for all manipulations including (but not limited to): pipetting, harvesting infected cells for RNA, injection into animals, infecting cell cultures. No open bench work!

SIGNAGE During work with viral particles, a biohazard warning sign must be posted on the door alerting personnel of the presence of AAV.

BIOHAZARD SHARPS CONTAINERS shall be available to dispose of sharps waste, including broken glass, needles, blades, etc.

CENTRIFUGATION must be performed in closed containers and using sealed rotors or safety cups to minimize the risk of aerosol generation. Samples must be placed into and/or removed from cups within a BSC.

VACUUM all vacuum lines must be fitted with a HEPA filter and a vacuum flask, containing the appropriate tuberculocidal disinfectant (e.g. 10% Bleach) in a volume sufficient to provide the recommended final concentration for that disinfectant when the flask is near full. At the end of the work session, aspirate a small volume of concentrated disinfectant through the vacuum tubing, into the vacuum flask. The vacuum flask must sit for a minimum time of 30 minutes prior to drain disposal.

VORTEXING must be done in BSC.

PIPETTING Aerosol resistant (filtered) tips must be used when pipetting.

SHARPS (ONE-TIME USE) All sharps should be immediately disposed of in to a sharps container (located within the BSC). Needles must never be recapped.

PRACTICES FOR ANIMAL INJECTIONS

1. When rodents are infected with AAV vectors, an Animal Biosafety Level - 1 (ABSL-1) area must be approved and used for the procedure. When one of the specific requirements, as described under Engineering Controls is met, all viral injections must take place in a certified Class II BSC. Animals will be considered an AAV hazard for 72 hours after exposure to AAV. After the 72 hour period during which animals are considered a hazard, animals will be transferred to clean prepared cages. All soiled cages will
be covered by filter tops and brought to the cage wash for full sanitation. Filter tops on hazard exposed cages will only be opened to allow dumping of soiled bedding under a HEPA filtered dump station. Concurrent approvals are needed from IBC and the University Animal Welfare Committee (UAWC). At the discretion of the IBC, a higher BSL may be requested, depending on the presence of helper virus and the gene insert.

2. The following information must be posted in the animal room.
   - A description of special housing required to ensure safety of animal facility personnel, such as ventilated cabinets or hoods.
   - A label on the animal cage indicating the biological materials to be administered to live animals. (i.e., AAV w/ or w/o helper virus)
   - The name of individual(s) responsible for handling the materials.

All sign templates are provided by OVR to the animal facility staff for this purpose. Additional precautions may be required as determined by the University Animal Welfare Committee.

CONTAINMENT/PERSOAL PROTECTIVE EQUIMENT

CONTAINMENT REQUIREMENTS: Biological Safety Level 1 facilities and equipment for work involving clinical and non-clinical specimens and/or non-culture procedures. If one of the specific requirements, as described under Engineering Controls is met, Biological Safety Level 2 practices must be followed.

PROTECTIVE CLOTHING: At minimum, gloves, closed toed shoes, and appropriate eye protection prior to working with any AAV. Lab coats are adequate for tissue culture manipulation. Additional precautions and procedures can be found in the UAWC’s standard operating procedures (SOPs) for AAV use in other species.

OTHER PRECAUTIONS: All activities with AAV must be conducted in a BSC (when a helper virus, oncogenic materials or injection is occurring in human or non-human primate tissues) and in combination with personal protective equipment. Centrifugation of infected or potentially infectious materials must be carried out in closed containers placed in sealed safety cups, or in rotors that are unloaded in a biological safety cabinet. The use of needles, syringes, and other sharp objects should be strictly limited. Open wounds, cuts, scratches, and grazes should be covered with waterproof dressings. Additional precautions should be considered with work involving animals or large scale activities.

SPILL PROCEDURES

1. Notify others working in the lab.
2. Get your spill kit and don new PPE (e.g. gloves, eye protection, and disposable coveralls).
3. Cover area of the spill with absorbent material and add freshly prepared 10 % Bleach, starting at the perimeter working inwards toward the center.
4. Allow 20 minutes of contact time. After 20 minutes wipe-up bleached material with paper towels or absorbent pads.
5. Dispose of materials as biological waste/regulated medical waste.
6. If any sharp objects or broken glass is contaminated with blood, remove objects with tongs or forceps and place in a sharps container. Never remove sharps/broken glass by hand.
7. Inspect the blood spill area closely; making sure that there is nothing missed and that the clean-up process is complete.
PREVENTING ACCIDENTAL EXPOSURES:
1. Employees should wash their hands with soap and warm water immediately after removal of gloves and other protective equipment.
2. Disinfect all reusable equipment.
3. Report all accidental exposures to your supervisor

EXPOSURE PROCEDURES

1. Contaminated PPE should be removed.
2. Proceed to the closest sink.
3. If the exposure involves broken/compromised skin, (needle sticks and/or sharps) the area should be immediately rinsed with cool running water and washed thoroughly with soap for 15 minutes.
4. After thoroughly rinsing the area, apply antiseptic and a clean dressing.
5. If the exposure involves the contamination of the mucus membrane(s), the area should be treated by flushing with cool water for 15 minutes at the nearest emergency eyewash station.
6. All Staff and students should notify their PI and Public Safety.

- Washington Sq. Notify the PI and Public Safety 8-2222 immediately
- Dental & 433 1st Ave must contact PI and PUBLIC SAFETY 8-9828 immediately
- NYUTSOE notify PI and PUBLIC SAFETY 646-977-3537 immediately or 8-2222 for after hour emergencies.

7. Seek medical attention by reporting to Student Health Center (students) or NYU Langone Medical Center (NYU Staff) for post exposure follow-up. Provide the medical provider with the following description:

- Biological material
- The route of exposure (e.g. needle stick or splash to eyes)
- Time and place of the incident
- Prior first aid provided

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<tr>
<th>Student Health Center</th>
<th>NYULMC Hospital (employees)</th>
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<tbody>
<tr>
<td>Washington Square</td>
<td>1 Park Avenue</td>
</tr>
<tr>
<td>726 Broadway</td>
<td>3rd Fl</td>
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<tr>
<td>3rd &amp; 4th Floors</td>
<td>New York, NY 10016</td>
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<td>New York, NY 10003</td>
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<tr>
<td>(212) 443-1000</td>
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<td>M-TU 10AM-8PM</td>
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<td>W-F 10AM-6PM</td>
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<td>SA 10AM-4PM</td>
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<tr>
<td><strong>THE PI/LAB SUPERVISOR IS RESPONSIBLE FOR IMMEDIATELY REPORTING AN EXPOSURE INCIDENT TO THE BIOSAFETY OFFICER.</strong></td>
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<th>Type of spill or exposure</th>
<th>Reporting time frame</th>
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<tr>
<td>Exposure to a Human or NHP materials, Risk Group 2 or 3 agents</td>
<td>Report immediately to the Biosafety Officer. Submit Incident Reporting Form* to the Biosafety Officer within 5 days of exposure.</td>
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<tr>
<td>Exposure to r-sNA materials</td>
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<tr>
<td>Large spill outside the BSC with a Human or NHP materials, Risk Group 2 or 3 agents</td>
<td>Incident Reporting Form must be submitted to the Biosafety Officer within 7 days of exposure.</td>
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Large spill outside the BSC with r-sNA materials

*Lab Incident Reporting form can be found on the Biosafety website

STORAGE AND DISPOSAL

**DISPOSAL:**

**Liquid waste:** must be aspirated into a vacuum flask containing 1/10 volume concentrated bleach or 1/40 volume Wescodyne. A common practice is to anchor the end of the vacuum tubing to the outside of the sash or frame of the Biosafety Cabinet. At the end of the work session, aspirate 25-50 ml of concentrated bleach through the vacuum tubing, into the vacuum flask. The vacuum flask must have a final concentration of at least 10% bleach, for a minimum time of 30 minutes prior to drain disposal. Liquid waste that is not aspirated must be treated with bleach, to a final concentration of at least 10%, in the hood, allowing a minimum time of 30 minutes to inactivate virus or autoclaved. A simple 500 ml bottle with 100 ml concentrated bleach may be suitable to collect non-aspirated liquid waste.

**Solid biohazardous waste:** Everything that contacts virus-containing solutions or vessels must be decontaminated or contained before exiting the biosafety cabinet. Solid waste can be collected in a biohazard bag or sharps container inside the Biosafety Cabinet. At the end of the work session, the biohazard bag will be closed, sprayed with 70% EtOH, and deposited into a biohazardous waste container.

Other solid waste such as culture vials, plates, plastic tubes, etc., are disposed of into an autoclave bag, autoclaved for 60 minutes at 121°C under 15lbs psi of steam pressure, sealed, and labeled, and placed in a 2 layered biohazardous box-bag unit for pickup.

**Sharps waste:** such as broken glass, pasteur pipets, razor blades, and needles, are disposed of into an approved biohazard sharps containers, autoclaved for 60 minutes, placed in a box-bag unit for pickup.

**STORAGE:** of AAV stocks must be in leak-proof secondary containers (i.e. freezer boxes) in a -80° freezer clearly marked with a warning label to indicate that AAV is present.

SUPPLEMENTAL REFERENCES

# TRAINING RECORD

**AGENT: ADENO-ASSOCIATED VIRAL VECTORS**

I have reviewed and understand the risks associated working with ADENO-ASSOCIATED VIRAL VECTORS. I understand that my signature below indicates I agree to comply and work safely with the said agent.

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