

Biosafety Level 2 Enhanced (BSL2+) for Laboratories Utilizing Viral Vectors

The following information applies to any work with Adenoviral vectors, Adeno-Associated Virus (AAV), Lentiviral vectors, and Retroviral vectors. This information may also be applicable to other viral vector systems. Please contact the Department of Biological Safety if you are using other vector systems.

Containment Level:

Work with these agents should be performed in a laboratory where consistent biosafety level 2 practices are adopted and rigorously followed and the facility requirements for BSL2 are met. Some BL3 practices are also adopted. This is often referred to as BL2+.

Laboratory Facility:

The Principal Investigator shall designate a laboratory that contains a handwashing sink, biological safety cabinet (BSC), incubator, centrifuge, microscope, and CO₂ source. It is preferable that this be an inner lab with 2 doors between the BSC and the hallway and restricted entry to the lab. Air shall flow from the hallway to this lab (negative to the hallway) and all air is exhausted outside the building, not recirculated. A sign stating that viral vectors are present, entry is restricted to authorized personnel, and doors are to remain closed should be posted on laboratory doors.

Personal Protective Equipment (PPE):

PPE required for work with viral vectors includes gloves and lab gown; gloves shall be pulled over the knit cuffs of a disposable lab gown which ties in the back and is liquid resistant (www.fishersci.com Wrap-around gowns with knit cuffs Catalog Number 19-999-344B). Double gloving should be considered, depending upon the procedures being performed. Standard laboratory safety dress code shall be followed (no shorts or open toed shoes, etc.). Prior to commencing any work with these agents, a protocol shall be approved by the Institutional Biosafety Committee (IBC).

Laboratory Practices:

- All experimental materials shall be handled with great care.
- Laboratory doors shall remain closed.
- No work with these viral vectors is permitted on the open bench. A biological safety cabinet (BSC) or comparable containment equipment shall be used for all manipulations including (but not limited to):
 - Pipetting,
 - Harvesting infected cells for nucleic acid, protein, etc.,
 - Loading and opening containers.
- Within the Biological Safety Cabinet (BSC):
 - Used plastic pipet tips and serological pipettes are to be placed in a plastic-lined cardboard box for decontamination and disposal.
 - This box is placed into an autoclave bag with any other biohazardous waste in the BSC. The bag is to be closed and then sprayed with a disinfectant approved for efficacy against the viral vector in use.
 - The disinfected bag is then placed into a larger clean autoclave bag, and subsequently autoclaved for 1 hour at 121°C or 250°F (15 lbs per square inch of steam pressure).
 - Biosafety cabinet surfaces shall be thoroughly wiped with disinfectant approved for efficacy against the viral vector in use.
- For aspiration, use a plastic vacuum flask with a second vacuum flask connected to it as a backup, with non-collapsible tubing capable of withstanding disinfection. To the second

vacuum flask attach a filter with both a hydrophobic and a HEPA filter component (www.fishersci.com Vacuum trap filters Catalog Number 09-744-75) so that no potentially infectious aerosols are aspirated into the house vacuum system or vacuum pump. These 3 items are attached in series from the vacuum source in the biosafety cabinet or a vacuum pump. If this set-up is placed outside the biosafety cabinet, it should be contained in a tray or pan.

- Undiluted bleach should be pulled into the aspirator flask using suction to decontaminate tubing. Final concentration in the aspirator flask when it is 2/3 full should be at least 10% bleach/0.5% NaOCl. The aspirator should be emptied when it is 2/3 full.
- Biohazard waste containers should be hard-sided with a foot operated lid, and easily decontaminated with liquid disinfectant. The autoclave bag should be folded over the rim so that only the inside of the bag is visible.
- All centrifugation shall be done in closed containers using sealed rotors or buckets.
- Sealed rotors and buckets shall be loaded and unloaded in the biosafety cabinet and disinfected with an appropriate disinfectant prior to being removed from the biosafety cabinet. A sturdy cart should be used to transport rotor between centrifuge and biosafety cabinet.
- Signs and labels shall be placed to indicate each area where viral vectors are used or stored (biological safety cabinets, incubators, refrigerators, laboratory entrance doors, etc.).
- Laboratory shall have negative air pressure relative to the hallway.
- Laboratory gowns should be water resistant and have cotton knit cuffs with back closure and wrap around ties.
- Effective germicides with appropriate contact times shall be utilized.
- Sharps shall be eliminated from experimental procedures. Do not use "sharps" to harvest virus pellet. No "sharps" (needles, glass Pasteur pipets) may be used with these cultures. Plastic aspiration pipets are a replacement for glass Pasteur pipets.
- Gloves shall be worn at all times when working with viral vectors. Remove gloves using the inside-out technique. Dispose of gloves into biohazard waste container which will be autoclaved. Wash hands immediately after removing gloves and before leaving work area. Never wear gloves outside of the laboratory, or touch doorknobs, telephones, personal belongings, etc. with gloved hands.
- All cultures, stocks, or materials used to manipulate or otherwise exposed to viral vectors shall be autoclaved prior to disposal (except sharps). Autoclave conditions to be met: 1 hour at 121°C or 250°F (15 lbs per square inch of steam pressure). The outside of sharps containers shall be decontaminated with liquid disinfectant before pick-up by Environmental Management for incineration.

Animal Use with Viral Vectors:

- Concurrent approvals are needed from UK Institutional Biosafety Committee (IBC) and UK Institutional Animal Care and Use Committee (IACUC) prior to commencing animal work with viral vectors.
- Viral vector stocks must be tested for the presence of [replication competent virus](#) prior to introduction into research animals.
- Animals shall be handled in a BSL2/ABSL2 area designated and approved for viral vector work.
- Precautions shall be taken not to create aerosols when emptying animal waste material, washing cages, or cleaning the room.
- Special training shall be given to all animal husbandry personnel by the PI or DLAR veterinarians or supervisors on the specific viral vectors in use. This training shall address the hazards associated with the work, required practices and procedures and proper handling of bedding, cage washing, and all other husbandry materials associated with the experiment.

- All necropsy shall be performed using BSL2/ABSL2 precautionary practices and procedures.
- Adenoviral Vectors and Animals: Infected animals may excrete (shed) adenovirus (especially the first 72 hours after infection). Only lab personnel or animal husbandry workers trained to handle animals infected with adenovirus should be responsible for animal husbandry practices during the first 72 hours following infection of the animal.

Accidental Exposure:

Eye exposure from splash or aerosol

Rinse a minimum of 15 minutes in eye wash or flush with water. Notify the Principal Investigator or Laboratory Supervisor, who will immediately contact Worker's Care at 1-800-440-6285 and direct the exposed employee to appropriate medical treatment at University Health Services, and to report the incident.

Needlestick, sharps exposure or non-intact skin exposure

Contaminated skin should be scrubbed for approximately 20 minutes using a 10% povidone iodine solution (such as Betadine®) and copious amounts of water. Notify the Principal Investigator or Laboratory Supervisor, who will immediately contact Worker's Care at 1-800-440-6285 and direct the exposed employee to appropriate medical treatment at University Health Services, and to report the incident.

Adenovirus Specific Information:

Adenovirus is a pathogen of respiratory and gastrointestinal mucous and eye membranes. Adenovirus (replication deficient and replication competent) can cause corneal and conjunctival damage. Eye protection (goggles) shall be worn when working with this agent/vector. Adenovirus (unlike HIV or herpes) is quite stable. After having been extracted with ether and/or chloroform, it can still be infective. The replication-defective virus may be complemented in vivo by endogenous adenovirus thereby causing the vector to become replication competent. Adeno-associated viral vectors shall be tested for presence of replication competent adenovirus prior to injection into animals. Adenovirus may be transmitted by droplet, aerosol, or injection. If experimental parameters make it impossible to work with adenovirus within a laminar flow biosafety cabinet, a respiratory protection program shall be in place and N95 fit-tested respirators shall be worn. Contact Occupational Health and Safety or the Biological Safety Officer for more information. Any of these symptoms may occur following adenovirus exposure: acute respiratory illness (cold like symptoms), pneumonia, conjunctival infection (red eye), or corneal inflammation leading up to scarification.

References:

Biosafety in Microbiological and Biomedical Laboratories-5th Edition
http://www.cdc.gov/OD/ohs/biosfty/bmbl5/BMBL_5th_Edition.pdf

NIH Guidelines for Research Involving Recombinant DNA Molecules
http://oba.od.nih.gov/oba/rac/guidelines_02/NIH_Gdlnes_Ink_2002z.pdf

University of Kentucky Biosafety Manual
http://ehs.uky.edu/hmm/biosafety_manual.html

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