

## Policy 110: Viral Vectors

### I. INTRODUCTION

Viruses and viral vectors are extremely valuable tools to the research community. Thus, it is important for users to understand the origins of these tools and potential implications of their use. The Viral Vector Summaries in this policy outline how to appropriately and safely use the most commonly used viral vectors at Dartmouth.

### II. VIRAL VECTOR BIOSAFETY

Viral vectors may infect not only experimental specimens but also laboratory researchers. Rendering an infectious virus replication-incompetent or otherwise attenuated lowers the risk of working with them, and later generation viral vector systems are generally safer than early generation systems. However, these improvements in safety and the increased commercial availability of viral vectors may result in a false sense of security and a decrease in practicing safe science. Furthermore, recombination events or contamination from wild-type virus can result in the presence of replication competent viruses in a population of replication deficient viral vectors. Special consideration should be given to viral vectors containing genes for products that regulate growth (oncogenes, tumor suppressors, growth factors, etc.), may have immunological effects (cytokines, etc.), are excreted, or may be toxic.

### III. RESEARCH OVERSIGHT

Viral vectors are regulated by the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines, 2019)*, which **requires** that all viral vector use must be reviewed and approved by the Institutional Biosafety Committee (IBC) **before** vectors are obtained and/or used. This review will include a risk assessment to determine the appropriate biosafety level, personal protective equipment, disposal methods, and possible need for replication competency testing. The biosafety levels listed in Section IX (p. 18) apply to replication incompetent viral vector systems only for *in vitro* and *in vivo* experiments. Additional biosafety precautions may be recommended or required by the IBC, and appropriate practices for work that falls outside the scope of this document will be determined in consultation with the biosafety officer and IBC.

### IV. TRAINING

All research personnel in labs that work with viral vectors must complete the Viral Vector Biosafety module on BioRAFT. Those who directly handle vectors should review this policy and must also receive hands-on training by their PI or designee before being allowed to work independently.

### V. VIRAL VECTOR SUMMARIES

This section discusses viral vectors derived from adenovirus, adeno-associated virus, baculovirus, Epstein-Barr virus, herpes simplex, lentivirus, Moloney murine leukemia virus, rabies, and poxviruses.



### A. Adenoviral Vectors (Ad)

**Background:** Adenoviruses are non-enveloped icosahedral viruses containing double-stranded DNA. Adenoviruses are infectious human viruses that often cause mild respiratory illness, pink eye or gastroenteritis. Rare cases of severe disease can occur, and its use as a genetic vector therefore requires the use of adequate containment equipment and practices. Particular care should be given to vectors containing genes that make products similar to those of the deleted adenovirus genes. Symptoms of respiratory illness caused by adenovirus infection range from the common cold syndrome to pneumonia, croup, and bronchitis. Patients with compromised immune systems are especially susceptible to severe complications of adenovirus infection that can cause more systemic diseases (e.g. hepatitis).

**Recombination:** The probability of producing replication competent adenovirus (RCA), although low, increases with each successive amplification. RCA is produced when adenoviral DNA recombines with E1- containing genomic DNA in HEK 293 cells. It is suggested to use early amplification stocks when production of additional quantities of adenovirus are needed.

Virus packaged by transfecting HEK 293 cells with adenoviral-based vectors is capable of infecting human cells. These viral supernatants could, depending on the gene insert, contain potentially hazardous recombinant virus. Similar vectors have been approved for human gene therapy trials, attesting to their potential ability to express genes *in vivo*. For these reasons, due caution must be exercised in the production and handling of any recombinant adenovirus.

**Biosafety Level:** BSL-2, all work performed in a biosafety cabinet.

**Animal Biosafety Level:** All animal procedures require pre-approval by the Dartmouth College Institutional Animal Care and Use Committee. Injections of rodents must be performed in a biosafety cabinet at ABSL-2. ABSL-2 housing is required post-injection/exposure of animals. For rodents that do not contain any human cells or tissues, then ABSL-2 containment is required for the first 48 hours, and the animals may be downgraded to ABSL-1 thereafter. The IBC may raise containment if deemed necessary. Animal bedding/cages are autoclaved from ABSL-2 animals.

**Disinfection/Deactivation:** Adenoviruses are unusually stable in chemical or physical agents and diverse pH conditions, allowing for prolonged survival outside of the body. Susceptible to 1% sodium hypochlorite, 2% glutaraldehyde, or 0.25% sodium dodecyl sulfate.

**Laboratory Hazards:** Ingestion; droplet exposure of the mucous membrane.

Laboratory Hazards	PPE
Exposure of mucus membrane (eyes, nose, mouth)	Safety goggles or full face shield, appropriate face mask
Injection	Safety needles, NEVER re-cap needle or remove from syringe
Aerosol inhalation	Appropriate respiratory protection
Direct contact with skin	Gloves, lab coat, closed shoes

**Treatment:** Most infections are mild and require no therapy or only symptomatic treatment. Because there is no virus-specific therapy, serious adenovirus illness can be managed only by treating symptoms and complications of the infection.



**B. Adeno-associated Viral (AAV) Vectors**

**Background:** Adeno-associated virus gets its name because it is often found in cells that are simultaneously infected with adenovirus. AAV are non-enveloped icosahedral viruses with a single stranded DNA genome. These are infectious human viruses with no known disease association. Some AAV types are common in the general population, and these viruses have the ability to integrate into the host genome.

The *NIH Guidelines* (Appendix B) state that "adeno- associated virus (AAV – all serotypes); and recombinant or synthetic AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the absence of a helper virus" can in most cases be handled at biosafety level 1 (BSL-1).

**Recombination:** AAV is dependent on the presence of wild type adenovirus or herpesvirus for replication; in the absence of these helper viruses, AAV may stably integrate into the host cell genome. Co-infection with helper virus triggers a lytic cycle. Wild type AAV integrates preferentially into human chromosome 19q13.3-qter; recombinant vectors lose this specificity and appear to integrate randomly, thereby posing a theoretical risk of insertional mutagenesis and cell transformation and tumor formation.

**Biosafety Level:** In absence of helper virus: BSL-1. In presence of helper virus: BSL-2, all work performed in a biosafety cabinet.

**Animal Biosafety Level:** All animal procedures require pre-approval by the Dartmouth College Institutional Animal Care and Use Committee. ABSL-1 housing; ABSL-2 housing in the presence of helper virus. The IBC may raise containment if deemed necessary. Animal bedding/cages are autoclaved from ABSL-2 animals.

**Disinfection/Deactivation:** Susceptible to 1% sodium hypochlorite, 2% glutaraldehyde, or 0.25% sodium dodecyl sulfate.

**Laboratory Hazards:** Ingestion, droplet exposure of the mucous membrane, direct injection; insertional mutagenesis possible but unlikely; integration and expression of oncogenes or potential oncogenes.

Laboratory Hazards	PPE
Exposure of mucus membrane (eyes, nose, mouth)	Safety goggles or full face shield, appropriate face mask
Injection	Safety needles, NEVER re-cap needle or remove from syringe
Aerosol inhalation	Appropriate respiratory protection
Direct contact with skin	Gloves, lab coat, closed shoes

**Treatment:** No specific treatment.



**C. Baculoviral Vectors**

**Background:** Baculoviruses are non-mammalian enveloped, circular DNA viruses that infect insects. Generally, non-genetically modified wild type baculoviruses are not capable of replicating in vertebrate cells and thus do not pose any inherent hazards to laboratory workers. However, more recent studies with the use of mammalian specific promoters have achieved expression of foreign genes in a wide variety of mammalian cell lines and primary cell cultures.

**Recombination:** Not known to occur in human cells.

**Biosafety Level:** Work is mostly done at BSL-1. Containment levels may be raised per IBC review if the vector is amphotropic and can infect human cells.

**Animal Biosafety Level:** All animal procedures require pre-approval by the Dartmouth College Institutional Animal Care and Use Committee. ABSL-1.

**Disinfection/Deactivation:** 1-10% sodium hypochlorite or 70% ethanol.

**Laboratory Hazards:** Direct contact, droplet exposure of the mucous membrane, direct injection.

Laboratory Hazards	PPE
Exposure of mucus membrane (eyes, nose, mouth)	Safety goggles or full face shield, appropriate face mask
Injection	Safety needles, NEVER re-cap needle or remove from syringe
Direct contact with skin	Gloves, lab coat, closed shoes

**Treatment:** No specific treatment, not known to be hazardous to humans.



**D. Epstein-Barr Viral (EBV) Vectors**

**Background:** Epstein-Barr virus, frequently referred to as EBV, is a member of the gamma herpesvirus family and one of the most common human viruses. EBV are enveloped, icosahedral viruses with a double-stranded linear DNA genome. The virus is found worldwide, and most people become infected with EBV sometime during their lives, most commonly causing infectious mononucleosis - acute viral syndrome with fever, sore throat, splenomegaly and lymphadenopathy. A few carriers of this virus may develop Burkitt's lymphoma or nasopharyngeal carcinoma. EBV is a transforming virus and is often used to produce immortalized cell lines and cause lymphoma in various animal models.

**Recombination:** Potential for recombination with a latent viral infection.

**Biosafety Level:** BSL-2; all work is performed in a biosafety cabinet.

**Animal Biosafety Level:** All animal procedures require pre-approval by the Dartmouth College Institutional Animal Care and Use Committee. Injections of rodents must be performed in a biosafety cabinet. ABSL-2 housing is required post-injection/exposure of animals. Animal bedding/cages are autoclaved from ABSL-2 animals.

**Disinfection/Deactivation:** 1% sodium hypochlorite, 70% ethanol, glutaraldehyde, or formaldehyde.

**Laboratory hazards:** Ingestion, accidental parenteral injection, droplet exposure of the mucous membranes, inhalation of concentrated aerosolized materials. Note that cell lines are often immortalized by transformation with EBV.

Laboratory Hazards	PPE
Exposure of mucus membrane (eyes, nose, mouth)	Safety goggles or full face shield, appropriate face mask
Injection	Safety needles, NEVER re-cap needle or remove from syringe
Aerosol inhalation	Appropriate respiratory protection
Direct contact with skin	Gloves, lab coat, closed shoes

**Treatment:** No specific treatment.



### E. Herpes Simplex Viral Vectors

**Background:** Herpes Simplex viruses are members of the alpha herpesvirus family and are enveloped, icosahedral, double-stranded linear DNA viruses. Herpesviruses include infectious human viruses such as herpes simplex virus type-1 (HSV-1), which is the most commonly used vector system. HSV-1 is common in the general population, but can cause encephalitis in rare cases; its utility as a vector system stems from its broad host cell range, ability to transduce neurons, and its large insert capacity. HSV is spread by direct contact with epithelial or mucosal surfaces.

Vectors derived from Herpes simplex virus (HSV) have some unique features. The vectors have a wide host range and cell tropism, infecting almost every cell type in most vertebrates that have been examined. In addition, the natural property of the virus to infect and establish latent infection indefinitely in post-mitotic neurons has generated substantial interest in using it to deliver therapeutic genes to the nervous system.

**Recombination:** All versions of HSV vectors are prone to recombination. Additionally, approximately 50% - 90% of adults possess antibodies to HSV type 1; 20% - 30% of adults possess antibodies to HSV type 2. This is a concern since reactivation from latency is not well understood. Infection by HSV vectors into latently infected cells could potentially reactivate the wild-type virus, or spontaneous reactivation of a latent infection could produce an environment where replication defective vectors could replicate.

**Biosafety Level:** BSL-2; all work is performed in a biosafety cabinet.

**Animal Biosafety Level:** All animal procedures require pre-approval by the Dartmouth College Institutional Animal Care and Use Committee. Injections of rodents must be performed in a biosafety cabinet. ABSL-2 housing is required post-injection/exposure of animals. Animal bedding/cages are autoclaved from ABSL-2 animals.

**Disinfection/Deactivation:** Susceptible to common disinfectants - 1% sodium hypochlorite, iodine solutions containing ethanol, 70% ethanol, glutaraldehyde, or formaldehyde.

**Laboratory Hazards:** Ingestion; accidental parenteral injection; droplet exposure of the mucous membranes of the eyes, nose, or mouth; inhalation of concentrated aerosolized materials.

Laboratory Hazards	PPE
Exposure of mucus membrane (eyes, nose, mouth)	Safety goggles or full face shield, appropriate face mask
Injection	Safety needles, NEVER re-cap needle or remove from syringe
Aerosol inhalation	Appropriate respiratory protection
Direct contact with skin	Gloves, lab coat, closed shoes

**Treatment:** Anti-viral drug therapy for symptoms.



## F. Lentiviral Vectors

**Background:** Lentiviruses are a subset of retroviruses, which are simple, enveloped single-stranded RNA viruses. Retroviruses are transmitted through direct exposure with bodily fluids, percutaneous exposures, or sexual contact. Lentiviruses have the ability to integrate into host chromosomes, to infect non-dividing cells, and have high mutation rates. These viruses cause multi-organ diseases characterized by long incubation periods, immune evasion, and persistent infections in their natural hosts. Acute infection with human lentiviruses can appear as non-specific “flu-like” and “mononucleosis-like” symptoms, including myalgia, diarrhea, nausea, vomiting, headache, weight loss and neurological symptoms.

**Types:** Lentiviral vector systems can include viruses of non-human/non-primate origin (feline immunodeficiency virus, equine infectious anemia virus) as well as simian viruses (simian immunodeficiency virus) and human immunodeficiency viruses (HIV).

**Pseudotyping:** Replacement of the lentiviral envelope glycoprotein with a heterologous envelope, such as VSV-g, provides a broad host-range for the vector in terms of host and cell type transmissibility.

**Vector Systems:** Most of the lentiviral vectors presently in use are HIV-derived vectors. The cis- and trans-acting factors of lentiviruses are often on separate plasmid vectors, with packaging being provided in trans. Second generation systems use two helper plasmids (one with *gag*, *pol*, *rev*, and *tat*; one with envelope protein) in addition to the transgene plasmid. Third generation systems offer more safety features, including the use of three or more helper plasmids to separate the packaging and gene transfer functions in addition to the transgene plasmid. These systems are self-inactivating and do not include *tat*. Fourth generation systems further split *gag* and *pol* onto separate plasmids to reduce recombination, while adding back some HIV genes to enhance transduction efficiency and transgene expression. The IBC strongly encourages the use of third or fourth generation systems where possible.

**Recombination:** Third and fourth generation vectors have been designed to significantly diminish the possibility for recombination to occur resulting in a wild-type infectious virus. The potential for generation of replication competent lentivirus (RCL) from HIV-1 based lentivirus vectors depends upon several factors, the most important of which are:

- i. the number of recombination events necessary to reassemble a replication competent virus genome
- ii. the number of essential genes that have been deleted from the vector/packaging system

Earlier vector systems such as 2<sup>nd</sup> generation vectors (three-plasmid vector systems) may have a higher potential for generation of RCL. Replication competency testing is required for 2<sup>nd</sup> generation systems and may be required in other situations. Please refer to *Replication Competency Testing of Retroviral and Lentiviral Vectors* (IBC Policy #111) for more information.

**Biosafety Level:** Please refer to [Biosafety Considerations for Research with Lentiviral Vectors](#). BSL-2 for non-human viruses, non-human pseudotyped viruses, or viruses that do not express transgenes with any known oncogenic potential or biological toxin; all work done in biosafety cabinet.

Enhanced BSL-2 for human viruses, amphotropic or VSV-g envelope pseudotyped viruses expressing transgenes with known oncogenic potential or a biological toxin. Enhanced BSL-2 containment means working with BSL-3 practices and procedures, including all work in the biosafety cabinet, increased



donning of PPE (2<sup>nd</sup> pair nitrile gloves, closed front disposable gown, mucous membrane protection), special attention to sharps use, and disinfection of all pipettes and other materials inside of a biosafety cabinet.

**Animal Biosafety Level:** All animal procedures require pre-approval by the Dartmouth College Institutional Animal Care and Use Committee. Injections of rodents must be performed in a biosafety cabinet at ABSL-2 with appropriate PPE. ABSL-2 housing is required post-injection/exposure of animals. Animal bedding/cages are autoclaved from ABSL-2 animals.

For rodents that do not or will not contain any human cells or tissues, then ABSL-2 containment is required for the first 72 hours, and then animals may be downgraded to ABSL-1 thereafter. The IBC may raise containment if deemed necessary.

**Disinfection/Deactivation:** Susceptible to many disinfectants - 1% sodium hypochlorite, 2% glutaraldehyde, formaldehyde, or 70% ethanol.

**Laboratory Hazards:** Direct contact with non-intact skin and mucous membranes of the eye, nose and mouth; accidental parenteral injection; ingestion; hazard of aerosols exposure unknown; insertional mutagenesis; integration and expression of oncogenes or potential oncogenes.

Laboratory Hazards	PPE
Exposure of mucus membrane (eyes, nose, mouth)	Safety goggles or full face shield, appropriate face mask
Injection	Safety needles, NEVER re-cap needle or remove from syringe
Aerosol inhalation	Appropriate respiratory protection
Direct contact with skin	Gloves, lab coat, closed shoes

**Treatment:** Specific measures for the opportunistic diseases that result from AIDS; multidrug treatment for HIV; anti-retrovirals; integrase inhibitors if treated immediately (within 72 hours of exposure; maximum benefit within first 2 hours of exposure).





### G. Moloney Murine Leukemia Virus (MMLV)

**Background:** MMLV is a retroviral subfamily, oncovirinae type C. MMLV are enveloped, icosahedral, diploid viruses with a single-stranded, linear RNA genome. MMLV integrates into the host genome and is present in infected cells as a DNA provirus. Cell division is required for infection.

**Pseudotyping:** The host range of recombinant MMLV vectors is dependent on the specificity of the viral envelope. The ecotropic env gene produces particles that infect only rodent cells. Amphotropic env gene allows infection of murine and nonmurine cells, including human cells. VSV-G envelope allows infection in a wide range of mammalian and non-mammalian cells. Chronic productive retroviral infection may allow insertional mutagenesis leading to cell transformation and tumor formation. The nature of a transgene or other introduced genetic element may pose additional risk with pseudotyped viruses.

**Recombination:** Possible. See info for Lentivirus.

**Biosafety Level:** BSL-1 (ecotropic vectors) or BSL-2 (amphotropic or VSV-g pseudotyped vectors containing biological toxin or gene with oncogenic potential); all work performed in biosafety cabinet

**Animal Biosafety Level:** All animal procedures require pre-approval by the Dartmouth College Institutional Animal Care and Use Committee. ABSL-1 (ecotropic vectors). ABSL-2 (amphotropic or VSV-g pseudotyped vectors containing biological toxin or gene with oncogenic potential). Injections of rodents must be performed in a biosafety cabinet at ABSL-2 with appropriate PPE. ABSL-2 housing is required post-injection/exposure of animals. Animal bedding/cages are autoclaved from ABSL-2 animals.

For rodents that do not or will not contain any human cells or tissues, then ABSL-2 containment is required for the first 72 hours, and then animals may be downgraded to ABSL-1 thereafter. The IBC may raise containment if deemed necessary.

**Disinfection/deactivation:** Susceptible to many disinfectants - 1% sodium hypochlorite, 2% glutaraldehyde, formaldehyde, or 70% ethanol.

**Laboratory Hazards:** *In vivo* transduction in humans appears to require direct injection with amphotropic or pseudotyped virus. Contact with feces or urine from infected animals for 72 hours post infection. Contact with tissues and body fluids of infected animals. Insertional mutagenesis; integration and expression of oncogenes or potential oncogenes.

Laboratory Hazards	PPE
Exposure of mucus membrane (eyes, nose, mouth)	Safety goggles or full face shield, appropriate face mask
Injection	Safety needles, NEVER re-cap needle or remove from syringe
Aerosol inhalation	Appropriate respiratory protection
Direct contact with skin	Gloves, lab coat, closed shoes

**Treatment:** No recommended treatment.

**H. Rabies Viral Vector (SAD-B19)**

**Background:** Rabies virus is a member of the Rhabdoviridae family and is a common zoonotic infection from bats and other wild mammals. Rabies is an enveloped, single-stranded, negative-sense RNA virus. Infection results in encephalitis or paralysis, and is often fatal. Due to its neuronal tropism, pseudotyped rabies virus vectors can be used to study neuronal trafficking or express endogenous genes efficiently in neurons.

**Rabies viral vectors:** SAD-B19 ( $\Delta$ G) is a modified rabies virus with the viral envelope B19 glycoprotein deleted, thereby rendering the virus unable to produce competent or infectious particles in transduced cells. As a result, the mutant virus cannot spread to any other surrounding cells from the originally infected cells. If the B19-glycoprotein is (intentionally) over-expressed as a transgene in a defined group of infected cells, the virus can trans-synaptically transport to adjacent cells only (single-step) and never go beyond.

**Recombination:** Since the rabies virus is a negative-strand RNA virus, it does not integrate into the cell genome and has no chance to produce a G protein RNA template. Therefore, there is essentially no risk to generate replication competent rabies virus.

**Pseudotyping:** The tropism of the viral vector may also be changed so that it cannot infect any mammalian cells except those that express a genetically-specified neuronal population transgene that encodes the envelope receptor (TVA) of this pseudotyped virus. Since mammalian neurons do not express TVA, the injected virus cannot infect wild-type human neurons. Alternatively, rabies vectors may be pseudotyped with a number of different envelope genes that increase its tropism, including *EnvA*, *VSV-g*, avian sarcoma leucosis virus glycoprotein, or *HIV env*.

**Disinfection/deactivation:** Susceptible to 70% ethanol, phenol, formalin, trypsin, some detergents.

**Biosafety Level:** BSL-2; all work performed in biosafety cabinet.

**Animal Biosafety Level:** All animal procedures require pre-approval by the Dartmouth College Institutional Animal Care and Use Committee. ABSL-1 for SAD-B19 vectors; others determined by IBC review.

**Laboratory Hazards:** Parenteral injection, droplet or aerosol exposure of mucous membranes or broken skin with infectious fluids or tissues.

Laboratory Hazards	PPE
Exposure of mucus membrane (eyes, nose, mouth)	Safety goggles or full face shield, appropriate face mask
Injection	Safety needles, NEVER re-cap needle or remove from syringe
Aerosol inhalation	Appropriate respiratory protection
Direct contact with skin	Gloves, lab coat, closed shoes

**Treatment:** Administration of rabies POST-exposure prophylaxis is a medical urgency. There is no established treatment for wild-type rabies once symptoms have begun, beyond supportive therapy. Treatment is likely unnecessary for replication incompetent rabies vectors such as SAD-B19.

**Vaccination:** Consultation is available to determine if vaccination is appropriate for personnel working with rabies vectors. Vaccination is not needed for working with SAD-B19 vaccine strain.



## I. Pox viruses/Vaccinia

**Background:** The poxviruses are the largest known DNA viruses and are distinguished from other viruses by their ability to replicate entirely in the cytoplasm of infected cells. Vaccinia is an enveloped double-stranded DNA virus that is highly stable and can cause severe infections in immunocompromised persons, persons with certain underlying skin conditions, or pregnant women. Such individuals should not work with vaccinia virus. Disease manifests itself as vesicular or pustular lesions, area of induration or erythema surrounding a scab or ulcer at inoculation site.

**Vaccination/Surveillance:** Vaccinia virus is the leading agent of laboratory-acquired poxvirus infections through percutaneous injury, ocular contact, and contact with infected animals or aerosols. Laboratory-acquired infections with standard, mutant, or bioengineered forms of vaccinia virus have occurred, even in previously vaccinated persons. Use of attenuated vaccinia strains can reduce risk. Dartmouth requires all personnel working with vaccinia to enroll in the Dartmouth Vaccinia Use and Surveillance Program and recommends vaccinia vaccination in some cases. Please refer to *Working with Vaccinia/Poxviruses* (IBC Policy #140) for further information.

**Highly Attenuated Vaccinia Strains:** Strains that are highly attenuated (see table below) are typically unable to replicate or replicate poorly in human cells. Vaccination is not recommended for laboratory experiments with highly attenuated vaccinia strains so long as no other orthopoxviruses are in use.

Highly Attenuated Vaccinia Strain	Biosafety Level	Derived from:	Vaccination Recommended?
MVA	1	Vaccinia virus (Ankara)	No
NYVAC	1	Vaccinia virus (Copenhagen)	No
TROVAC	1	Fowlpox virus	No
ALVAC	1	Canarypox virus	No

**Non-Highly Attenuated Vaccinia Strains:** A vaccinia strain is considered non-highly attenuated if the virus maintains its capacity to replicate productively in mammalian cells (see the table below); pre-exposure vaccination can prevent or minimize the impact of accidental laboratory exposure. The CDC recommends vaccination every 10 years for laboratory workers in the United States who have any contact with non-highly attenuated vaccinia strains. However, individuals who are pregnant; breastfeeding; have skin conditions such as eczema or atopic dermatitis; or those with altered immune systems are at increased risk from the vaccine, and should not be vaccinated and should not work with the virus.

Non-Highly Attenuated Vaccinia Strain	Biosafety Level	Vaccination Recommended?
WR (Western Reserve, mouse neuroadapted derivative)	2	Yes
NYCBOH (New York City Board of Health)	2	Yes
Copenhagen	2	Yes
Temple of Heaven	2	Yes
Lister	2	Yes



**Biosafety Level:** BSL-2 (unless in highly attenuated strains, see table above); all work in biosafety cabinet.

**Animal Biosafety Level:** All animal procedures require pre-approval by the Dartmouth College Institutional Animal Care and Use Committee. Injections of rodents must be performed in a biosafety cabinet at ABSL-2. ABSL-2 housing is required post-injection/exposure of animals. Animal bedding/cages are autoclaved from ABSL-2 animals.

**Disinfection/deactivation:** Susceptible to 1% sodium hypochlorite, 2% glutaraldehyde, or formaldehyde.

**Laboratory Hazards:** Ingestion, parenteral injection, droplet or aerosol exposure of mucous membranes or broken skin with infectious fluids or tissues.

Laboratory Hazards	PPE
Exposure of mucus membrane (eyes, nose, mouth)	Safety goggles or full face shield, appropriate face mask
Injection	Safety needles, NEVER re-cap needle or remove from syringe
Aerosol inhalation	Appropriate respiratory protection
Direct contact with skin	Gloves, lab coat, closed shoes

**Treatment:** Vaccinia immunoglobulin and an antiviral medication may be of value in treating complications.



## VI. EXPOSURE/INCIDENT RESPONSE AND REPORTING PROCEDURES

### A. Personnel Exposure

#### i. Exposure Examples

- Needlesticks or other percutaneous injuries from a contaminated sharp item
- Splashes to mucous membranes (eyes, nose, mouth)
- Bites/scratches from animals that have been exposed to any recombinant or synthetic nucleic acid material, whether or not the exposure leads to illness

#### ii. Immediate Response

- **SKIN** exposure: Immediately remove contaminated personal protective equipment or clothing and wash the contaminated area with an iodine solution or antibacterial soap and copious water for 15 minutes.
- **EYE** exposure: Flush the eye with water for at least 15 minutes at an eyewash station.

#### iii. Notify PI or supervisor. If PI/supervisor is not available, immediately proceed to next step.

#### iv. Medical Treatment

- During work hours, report to Occupational Medicine at DHMC between 7:30am-4:30pm (DHMC Faulkner Building, Level 4, near parking garage). Phone: (603) 653-3850.
- After hours and weekends: report to DHMC Emergency Room.
- If transport assistance is needed or injury requires emergency treatment, contact Safety and Security at 6-3333 or (603) 646-4000 from cell phone.
- If emergency, call 911.

#### v. Reporting

- Notify the Biological Safety Officer (BSO) immediately.
- The BSO will investigate the incident and notify the IBC Chair and EHS Director.
- The PI or worker will complete a Biohazard Incident Report form and submit it to the BSO within 24 hours, and submit an Employee Injury Form to Risk Management within 24 hours.
- If the IBC Chair and BSO determine that the incident involves (non-exempt) recombinant or synthetic nucleic acid (r/sNA molecules), the BSO will submit an NIH incident report to the NIH Office of Biotechnology Activities within 30 days. Incidents occurring in BSL-2 laboratories resulting in an overt exposure will be immediately reported to NIH OBA.
- Office of Risk Management and the Vice Provost for Research will also receive a copy of the incident report.



## **B. Environmental Release Procedures**

### i. Examples:

- Significant spill or release any r/sNA molecules outside of containment equipment
- Theft, loss, or release of any r/sNA molecules to the environment, including escape or improper disposal of a transgenic animal

### ii. Immediate Response

- See Section VII (p. 15) for spill response procedures.

### iii. Reporting

- Notify the BSO immediately via email or phone if not already contacted for assistance with spill-clean up.
- If the IBC Chair and BSO determine that the incident involves (non-exempt) r/sNA molecules, the BSO will submit an NIH incident report to the NIH Office of Biotechnology Activities within 30 days. Incidents occurring in BSL-2 laboratories resulting in an overt release will be immediately reported to NIH OBA.

## **C. Use of r/sNA Molecules Without Approval from the IBC**

Research using recombinant or synthetic nucleic acid molecules as defined above and as described in Sections III-A, -B, -C and -D of the *NIH Guidelines* must be approved by the IBC prior to initiation.

### i. Reporting

- Failure to obtain IBC approval prior to initiation of r/sNA research must be reported to the BSO immediately.
- BSO will prepare a detailed report for review by the IBC Chair and Vice Provost for Research. The report will be sent to the NIH OBA within 30 days.

EHS Office Phone: (603) 646-1762

[ehs@dartmouth.edu](mailto:ehs@dartmouth.edu)

[biosafety@dartmouth.edu](mailto:biosafety@dartmouth.edu)



## VII. SPILL RESPONSE

### A. *Preparing for Spills*

Most spills involving BSL-2 organisms and viruses and r/sNA molecules can be handled by researchers. Supplies to clean a spill appropriately must be available in any lab that works with or stores biohazardous materials.

### B. *Recommended Supplies*

#### i. Disinfectant

- Use appropriate disinfectant against the agents of concern (refer to individual viral vector descriptions), such as a fresh 1:10 bleach solution. Alcohol is not recommended for larger spills due to flammability concerns.

#### ii. Personal Protective Equipment (PPE) (minimally)

- Lab coat
- Gloves
- Face shield

#### iii. Other

- Sharps container for broken glass
- Absorbent pads
- Tongs
- Autoclave bags

### C. *Small spills*

Wipe up spill with a disinfectant-soaked paper towel and clean the surface with a suitable disinfectant. Allow disinfectant 20 minutes of contact time before removal.

### D. *Large Spills*

#### i. Spills outside of a containment device, i.e., the spill is not inside of a Biological Safety Cabinet (BSC), centrifuge, or other lab equipment

- Close off spill area to traffic, and notify coworkers.
- If the spill involved an aerosol, instruct all occupants to leave the room for 30 minutes to allow aerosols to settle.
- Place a sign on the door warning staff not to enter the room due to a spill.
- Remove contaminated lab coat or clothing and wash exposed skin.
- Put on clean gloves and lab coat.
- Prepare enough volume of a 1:10 dilution of chlorine bleach or other approved disinfectant to saturate the contaminated area.
- Contain the spill with paper towels or other absorbent pads.
- Flood the spill area with disinfectant. Leave on for 20 minutes.
- Push the absorbent material at the edge of the spill into the spill's center. Add more paper towels as needed.
- If glass is present, use tongs or forceps and a dustpan to remove pieces and place into a biohazard sharps container.
- Discard the paper towels into an orange autoclave waste bag.



- Using clean paper towels and a disinfectant, wipe all surfaces that may have come in contact with the spilled material. Discard any disposable PPE into an orange autoclave waste bag.
- Wash hands thoroughly.
- Notify Principal Investigator or Supervisor and the BSO.

ii. Spills inside of a Biological Safety Cabinet (BSC)

- Leave BSC on.
- Follow steps 4 through 15 above. Do not use 70% ethanol as it evaporates too quickly to allow adequate surface contact time.
- If the cabinet has a catch basin beneath the work surface and the spill resulted in liquids flowing into this area, more extensive decontamination is required.
- Ensure the drain valve under the cabinet is closed.
- Pour disinfectant onto the work surface and through the front and rear grilles into the drain pan. Allow 20 minutes contact time.
- Absorb spilled fluid-disinfectant from work surface with paper towels and discard in an orange autoclave bag.
- Prepare to empty drain pan. Place fresh disinfectant solution into a collection vessel. Attach flexible tubing to the drain valve. The tube should be of sufficient length to allow the open end to be submerged in the collection vessel to minimize aerosol generation.
- Open the drain valve and empty the drain pan into the collection vessel containing disinfectant. Flush the drain pan with water and remove the flexible tubing. Manage contaminated materials as if they are infectious.
- Remove protective clothing used during cleanup and place in a biohazard bag for autoclaving.
- Wash hands thoroughly.
- Notify Principal Investigator or Supervisor and the BSO to determine if vapor/gas decontamination of the cabinet and filters is necessary.
- Run BSC at least 10 minutes after cleanup, before resuming activity in the cabinet.

iii. Spills Inside of a Centrifuge

Spills or breakage of containers inside of an operating centrifuge pose a serious potential for exposure due to the creation of aerosols. If a primary container has broken in a centrifuge without a closed rotor or bucket, immediately suspend use, notify lab staff and PI and request assistance from the Biosafety Officer.

For suspected or confirmed spills/breakage in any centrifuge, wait at least 30 minutes after the centrifuge has stopped operating to initiate clean up.

- Put on lab coat, gloves and a face shield prior to opening centrifuge. Open carefully to assess the damage.
- If the spill is contained within a closed cup, bucket or rotor, spray the exterior with disinfectant and allow at least 20 minutes of contact time. Remove the carrier to the nearest biosafety cabinet (BSC). If a biosafety cabinet is not available, close the centrifuge; post a sign to indicate it cannot be used. Notify the PI and Biosafety Officer for assistance.
- If a BSC is available, gather supplies needed, such as a sharps container for broken glass and bins filled with disinfectant and place into the BSC. Use forceps to remove broken glass and place directly into sharps container. Carefully remove any unbroken tubes and place into a bin filled with disinfectant for 20 minutes. Wipe carrier/bucket with disinfectant.
- After disinfection, the carrier, bucket or rotor should be washed with a mild soap and water.
- Spray the interior of the centrifuge chamber with a disinfectant, let sit for 20 minutes and then wipe down.





- Dispose of all clean-up materials (except sharps) in orange autoclave bags. Dispose of sharps in biohazard sharps containers.
- Remove protective clothing and thoroughly wash hands.

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## VIII. RESOURCES

- *Biosafety Considerations for Research with Lentiviral Vectors*. National Institutes of Health Recombinant DNA Advisory Committee 2006  
[https://osp.od.nih.gov/wp-content/uploads/Lenti\\_Containment\\_Guidance.pdf](https://osp.od.nih.gov/wp-content/uploads/Lenti_Containment_Guidance.pdf)
- *Biosafety in Microbiological and Biomedical Laboratories*, 6<sup>th</sup> ed. Centers for Disease Control and Prevention and National Institutes of Health, 2020 June  
<https://www.cdc.gov/labs/BMBL.html>
- *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)*. National Institutes of Health Office of Science Policy, 84 FR 17858, 2019 April  
<https://osp.od.nih.gov/biotechnology/nih-guidelines/>
- Policy 111: Replication Competency Testing of Retroviral and Lentiviral Vectors. Dartmouth IBC 2022  
[http://www.dartmouth.edu/~ehs/biological/policies\\_sops.html](http://www.dartmouth.edu/~ehs/biological/policies_sops.html)
- Policy 140: Working with Vaccinia/Poxvirus. Dartmouth IBC 2022  
[http://www.dartmouth.edu/~ehs/biological/policies\\_sops.html](http://www.dartmouth.edu/~ehs/biological/policies_sops.html)



**IX. SUMMARY OF BIOSAFETY LEVELS**

Viral Vector	Biosafety Level		Additional Precautions
	<i>in vitro</i>	<i>in vivo</i>	
Adenovirus <b>NO</b> human materials	BSL-2	ABSL-2 48hrs, then ABSL-1	All BSL-2 work done in biosafety cabinet, transport specimen in sealed, labeled secondary container, dispose of as biohazardous waste
Adenovirus <b>WITH</b> human materials	BSL-2	ABSL-2	
Adeno-associated virus (AAV) <b>NO</b> helper virus	BSL-1	ABSL-1	
Adeno-associated virus (AAV) <b>WITH</b> helper virus	BSL-2	ABSL-2	All work done in biosafety cabinet, transport specimen in sealed, labeled secondary container, dispose of as biohazardous waste
Baculovirus	BSL-1	ABSL-1	
Epstein-Barr Virus	BSL-2	ABSL-2	All work done in biosafety cabinet, transport specimen in sealed, labeled secondary container, dispose of as biohazardous waste
Herpes Simplex Virus	BSL-2	ABSL-2	All work done in biosafety cabinet, transport specimen in sealed, labeled secondary container, dispose of as biohazardous waste
Lentivirus <i>non-human viruses or non-human pseudo-typed viruses with no oncogenic potential or biotoxin expression</i>	BSL-2	ABSL-2 72hrs, then ABSL-1	All work done in biosafety cabinet, transport specimen in sealed, labeled secondary container, dispose of as biohazardous waste
Lentivirus <i>human viruses or human pseudo-typed viruses and/or those with oncogenic potential or biotoxin expression</i>	Enhanced BSL-2 per IBC	ABSL-2	Enhanced BSL-2 work uses BSL-3 practices - increased PPE, all work in biosafety cabinet, needle protective devices, transport specimen in sealed, labeled secondary container, dispose of as biohazardous waste
MMLV <i>ectotropic</i>	BSL-1	ABSL-1	
MMLV <i>amphotropic and those with oncogenic potential or biotoxin expression</i> <b>NO</b> human materials	BSL-2	ABSL-2 72hrs, then ABSL-1	All work done in biosafety cabinet, transport specimen in sealed, labeled secondary container, dispose of as biohazardous waste
MMLV <i>amphotropic and those with oncogenic potential or biotoxin expression</i> <b>WITH</b> human materials	BSL-2	ABSL-2	All work done in biosafety cabinet, transport specimen in sealed, labeled secondary container, dispose of as biohazardous waste
Rabies SAD-B19	BSL-2	ABSL-1	
Vaccinia (MVA, NYVAC, TROVAC, ALVAC)	BSL-1	ABSL-1	
Vaccinia (WR, NYCBOH, Copenhagen, Temple of Heaven, Lister)	BSL-2, Enhanced BSL-2 per IBC	ABSL-2	Vaccination may be required. Enhanced BSL-2 work uses BSL-3 practices - increased PPE, all work in biosafety cabinet, needle protective devices, transport specimen in sealed, labeled secondary container, dispose of as biohazardous waste