

Vaccinia Virus and Viral Vectors

Background

Vaccinia virus is a member of the family Poxviridae, which are enveloped viruses with double stranded DNA genomes. These viruses replicate in the cytoplasm of mammalian cells because they do not require host replication machinery found in the nucleus. This trait allows poxviruses to replicate in enucleated cells. Vaccinia virus has a large genome, which allows for large inserts (up to 25 kb of foreign DNA) and a high level of DNA expression from prokaryotes or eukaryotes due to being regulated by a strong poxviral promoter. The foreign genes are stably inserted into the viral genome, allowing for efficient replication and expression including proper post translational modification in the infected cell. While transduction with the viral vector will be transient, there will be high levels of expression of the transgene.

Vaccinia virus is a human pathogen, and while it is the strain of poxvirus used for the small pox vaccine, it can cause flu-like symptoms in healthy individuals and more serious complications in immunocompromised individuals. Vaccinia virus is transmittable to others if contact with the vaccination site or area of infection occurs. Recombinant vaccines that utilize highly attenuated strains such as Modified Vaccinia virus Ankara (MVA) and NYVAC have been created to treat diseases. These attenuated strains are replication deficient and are recommended for use as vectors to replace wild type vaccinia virus. The primary safety concern with vaccinia viral vectors is the transgene. Examples of high risk transgenes are those that encode a toxin or are immunomodulatory. Also, when replication deficient strains are used in the presence of other orthopox viruses, there is a possibility of recombination to create replication competent viral vectors with the genes of interest. This is a safety concern that must be considered when assessing the biosafety level when working with these types of vectors.

Symptoms of Exposure

The classical symptom of a poxvirus infection is a vesicular or pustular lesion on the skin at the inoculation site. Serious complications can result in immunocompromised persons or persons with eczema.

Modes of Transmission

Vaccinia virus can cause infection through ingestion, parenteral injection, absorption through broken skin, droplet or aerosol exposure of mucous membranes with infectious fluids or tissues. An unvaccinated person can become infected through contact with a lesion or broken skin of an infected or vaccinated individual.

Host Range

Vaccinia virus can infect all mammalian cell types.

Environmental Stability

Poxviruses have a high resistance to environmental drying, surviving close to 40 weeks outside of the host.

Approvals

Experiments using vaccinia virus and vaccinia viral vectors require IBC approval before initiation of experiments.

Test Methods for Replication Competent Virus

Not applicable

Laboratory Practices

Generally, vaccinia virus is classified as a **Biosafety Level 2** (BSL-2) organism. Vaccinia virus requires BSL2 practices and procedures for all work with the virus and Animal Biosafety Level - 2 practices and procedures for all animal manipulations. Attenuated strains that are considered replication deficient, such as MVA and NYVAC can be handled at BSL-1 if no other orthopox viruses are present, but a risk assessment should be performed to determine potential hazards.

At the discretion of the IBC, experiments may need to be conducted at Biosafety Level -3 (BSL3). In the IBC application, the PI must justify that the gene to be expressed is not particularly harmful, and include citations to support these statements.

1. No work with vaccinia virus is permitted on the open bench.
2. A certified Class II biosafety cabinet that has been inspected within the last 12 months must be used for all manipulations including (but not limited to):
 - ◆ Pipetting
 - ◆ Harvesting infected cells for RNA
 - ◆ Purification of virus
 - ◆ Infection of cell culture
 - ◆ Infection of animals
3. Centrifugation must be done in closed containers with **sealed rotors or safety cups**. Safety cups are to be opened inside the biosafety cabinet.
4. All vacuum lines must be fitted with a HEPA filter (an example is the "Vacushield™" inline hydrophobic filter, Product # 4402 from Gelman Science , Millex FH vacuum line protector Millipore (Fisher) cat # SLFH05010, or "HEPA-VENT™" inline hydrophobic filter, Catalog # 6723-5000 from Whatman).
5. All laboratory staff working with or supervising work with vaccinia virus must be made aware of the hazards associated with the work, required safety practices and procedures, and proper handling of the agent, as well as be current on required laboratory safety and biosafety training requirements.
6. Animal carcasses must be placed in autoclave bags and be designated for infectious waste disposal.
7. Special training must be given to all animal husbandry personnel on vaccinia virus, 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. This training would be provided by animal facility supervisors in consultation with REHS.

8. Signs and labels must be placed to indicate each area where vaccinia virus is 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.
9. All work and manipulations of vaccinia virus must be conducted in a certified Class II biological safety cabinet. If there are extenuating circumstances or a biosafety cabinet that is unavailable, please contact REHS (at the numbers listed at the end of this SOP) as additional precautions may be required.

Personal Protective Equipment

1. Disposable gloves.
2. Disposable gown or equivalent when introducing vector into animals or performing necropsies. Lab coats are adequate for tissue culture manipulations.
3. Eye Protection.

Vaccination

1. It is recommended that laboratory workers handling replication competent vaccinia virus or other poxviruses (as applicable) and animal care staff handling replication-competent vaccinia-infected or contaminated animals receive the ACAM2000 vaccine. Vaccination is recommended before work commences and at least every 10 years if potential occupational exposure continues. Vaccination is not recommended for any work handling replication deficient vaccinia viruses or vaccinia viral vectors.
2. Laboratories which use this pathogen and Comparative Medicine Resource facilities in which it may be handled may choose to have an informational session from the Campus Employee Health Service/Occupational Medicine Service regarding vaccination before work commences with this virus. In addition, all faculty and staff at Rutgers University who may handle or potentially be exposed to this pathogen may go to the Campus Employee Health Service/Occupational Medicine Service for individualized counseling. Students may go to the Student Health Service.

Instructions in the Event of Employee Exposure

◆ EXPOSURE FROM SPLASH OR AEROSOLS – INHALATION

Report the incident to your supervisor and refer to the Rutgers Emergency Action Plan for further instructions. The supervisor should submit an incident report through <https://MyREHS.rutgers.edu> to document the event.

◆ EXPOSURE FROM SPLASH OR AEROSOLS – EYE CONTACT, SKIN AND/OR MUCOUS MEMBRANE

Rinse a minimum of 15 minutes in eye wash or flush area with water, report the incident to your supervisor and refer to the Rutgers Emergency Action Plan posted in the lab for further instructions. The supervisor should submit an incident report through <https://MyREHS.rutgers.edu> to document the event.

◆ NEEDLESTICK AND/OR SHARPS EXPOSURE

Contaminated skin should be thoroughly scrubbed for several minutes with soap or a 10% povidone solution (Betadine) and copious amounts of water. Report the incident to your supervisor and REHS immediately after scrub. Seek medical attention at Campus Employee Health Services/Occupational Medicine Services. After hours exposures should report to the closest Emergency Room. The supervisor should submit an incident report through <https://MyREHS.rutgers.edu> to document the event.

◆ EMERGENT EXPOSURES

For situations in which exposure to vaccinia virus occurred and medical treatment is an emergency, personnel should report to the Emergency Room, and ensure their supervisor completes incident report through <https://MyREHS.rutgers.edu> to document the event.

Decontamination

The most effective disinfectant against vaccinia virus is a 1:10 sodium hypochlorite (bleach) solution that is made fresh daily.

- ◆ To make this solution, dilute 1 part bleach to 9 parts tap water.
- ◆ Ensure a 15 minute contact time.
- ◆ Use this disinfectant for treatment of reusable equipment, surfaces, and liquid waste (final volume 1% bleach).

Disinfectant alternatives include phenolics, and 70% ethanol.

Autoclaving for 1 hour at 121°C or 250°F (15 lbs psi of steam pressure).

- ◆ Use this disinfection method for reusable equipment, liquid waste or solid waste.

Animal Practices

1. When animals are infected with vaccinia virus, an Animal Biosafety Level - 2 (ABSL-2) area must be used and approved by the animal facility staff and REHS for the procedure. Concurrent approvals are needed from the Institutional Biosafety Committee (IBC) and the Institutional Animal Care and Use Committee (IACUC).
2. All bedding, waste and animals shall be treated as biohazardous. Cage changing and husbandry must be performed according to the hazard sign provided by REHS. All waste must be decontaminated by autoclaving or chemical disinfection prior to disposal.
3. Animal carcasses must be placed in autoclave bags and be designated for infectious waste disposal.
4. All necropsies must be performed in a designated room using animal BSL-2 practices and procedures.
5. The following information must be posted on the door of the animal room. REHS will provide a sign template to the animal facility staff for this purpose.

- ◆ A description of special housing required to ensure safety of animal facility personnel, such as ventilated cabinets or filtered cages.
- ◆ A label on the animal cage indicating the hazardous materials to be administered to live animals. (i.e., vaccinia virus vector)
- ◆ The name of individual(s) responsible for handling the materials (i.e., Drs. X, Y and Z and Technicians A and B as per protocol #00000) and emergency contact information
- ◆ A description of how to handle animals, carcasses, and contaminated cages and bedding

References

- ◆ CDC-BMBL, 5th ed., www.cdc.gov/od/ohs/biosfty/bmbl5/BMBL_5th_Edition.pdf
- ◆ Stanford University, "Working with Viral Vectors," <https://ehs.stanford.edu/topic/biosafety-biosecurity/viral-vectors>
- ◆ Young, L.S., Searle, P.F., Onion, D., and V. Mautner. 2006. "Viral gene therapy strategies: from basic science to clinical application." J. of Pathology. 208:299-318.
- ◆ Braun, A. 2006. "Biosafety in Handling Gene Transfer Vectors." Current Protocols in Human Genetics. 12.1-12.18.
- ◆ Petersen BW et al. Use of vaccinia virus smallpox vaccine in laboratory and health care personnel. MMWR 2016;65(10):257-62.

Standard Operating Procedures

Acknowledgement Page

I, _____, have read the SOP for working with _____ Viral Vector. The following people will be conducting experiments using these vectors. The staff members know where to find a copy of this SOP in the laboratory and they understand the hazards and safe work practices as detailed therein.

Name	Job Title	Initials

Principal Investigator (print): _____

Principal Investigator (Signature): _____