

Poxvirus/Vaccinia Virus Vectors

Background

Poxviruses are the largest DNA viruses and can 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 vectors can accept up to 25 kb of foreign DNA, which is useful for expressing large genes from either prokaryotes or eukaryotes. 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.

Vaccinia virus is a human pathogen, causing disease in immunocompromised individuals and is the strain of poxvirus used for the smallpox vaccine. Vaccinia virus is transmittable to others if contact with the vaccination site or area of infection occurs. Vaccinia virus has been used to create recombinant vaccines to treat diseases and attenuated strains (NYVAC) have been created. NYVAC is missing 18 genes, rendering it less pathogenic. There are strains that are replication incompetent and these strains are recommended for use as vectors to replace wild type vaccinia virus.

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.

Host Range

Vaccinia virus can infect all mammalian cell types.

Approvals

Experiments using vaccinia virus require local IBC approvals and an informational session regarding vaccination by [Campus Employee Health Services/Occupational Medicine Services](#) before initiation of experiments. All personnel not wishing to receive the vaccination must sign a declination waiver.

Test Methods for Recombinant Virus-QC Tests

Not applicable because this virus is used as a replicating vector

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. It is recommended that laboratory workers handling vaccinia virus or other poxviruses (as applicable) receive the Dryvax vaccination. This applies to animal care staff handling vaccinia infected animals. All persons at UMDNJ who may handle this pathogen are required to have an informational session from the [Campus Employee Health Services/Occupational Medicine Services](#) regarding vaccination before work commences with this virus.

Poxvirus/Vaccinia Virus Vectors: Standard Operating Procedures

STANDARD ♦ OPERATING ♦ PROCEDURES

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 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 EOHSS.
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.

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. Goggles and/or face shield.
4. 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 is unavailable, please contact EOHSS (at the numbers listed at the end of this SOP) as additional precautions may be required.

Poxvirus/Vaccinia Virus Vectors: Standard Operating Procedures

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 Emergency Response Guide flip chart 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 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](#). Refer to Emergency Response Guide flip chart posted in the lab for after-hours exposure. The supervisor should submit an incident report through <https://MyREHS.rutgers.edu> to document the event.
- ♦ **EMERGENT EXPOSURES**
For situations in which exposure to AAV 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% sodium hypochlorite (bleach) solution that is made fresh daily.

- ♦ To make this solution, dilute 1 part Clorox to 5 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 infected with vaccinia virus shall be treated as biohazardous and handled in a biosafety cabinet. After all animals are removed from their primary enclosure immediately autoclave the empty cage. After autoclaving, dump the cage contents into medical waste and begin cleaning the cage for re-use. All waste must be decontaminated by autoclaving or chemical disinfectant prior to disposal

Poxvirus/Vaccinia Virus Vectors: Standard Operating Procedures

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 in the animal room. EOHSS 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 the hazardous materials are to be used in the protocol. See the following example: *“The protocol will be conducted at Biosafety Level 2 (BSL2) and Animal Biosafety Level 2 (ABSL2). Staff will use Class II biosafety cabinets for procedures that have the potential to generate aerosols, splash or spray. Personnel working on the protocol will be trained in the hazards of working with the recombinant vaccinia virus vectors and the measures they need to take to protect themselves. They will wear lab coats or gowns, protective gloves and protective eyewear for all aspects of the protocol.”*

References

CDC-BMBL, 5th ed., www.cdc.gov/od/ohs/biosfty/bmb15/BMBL_5th_Edition.pdf

Stanford University, “Working with Viral Vectors,”

http://www.stanford.edu/dept/EHS/prod/researchlab/bio/docs/Working_with_Viral_Vectors.pdf

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.

S

Vaccinia Virus Vectors: Standard Operating Procedures

Acknowledgement Page

I, _____, have read the SOP for Vaccinia Virus Vectors. 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): _____

S

Contact Information:



Rutgers Environmental Health and Safety
27 Road 1, Building 4086
Phone ♦ 848-445-2550
Fax ♦ 732-445-3109
Website ♦ <http://rehs.rutgers.edu>
Protocols ♦ <http://myrehs.rutgers.edu>