

Rabies Virus and Viral Vectors

Background

Rabies virus is a member of the family Rhabdoviridae, which are enveloped viruses with negative-sense, single-stranded RNA genomes. Rabies virus is neurotropic, inducing encephalitis and paralysis, and is often fatal in mammals. Due to the neurotropism of the virus created by the viral glycoprotein (G), it is an appealing model for neuronal tracing. Rabies viral vectors lack the G protein, which abolishes the ability to create competent viral particles after initial infection as well as the ability to spread trans-synaptically. This type of vector can infect cells normally, as the G protein has no role in transcription or replication. The vector can still express the remaining genes, as well as the transgene, and replicate inside the initially infected cells. However, the vector has no means of producing G protein and thus cannot infect new cells. The virus is able to be pseudotyped to express different envelope proteins that increase cell tropism. Additional modifications allow the viral vectors to only infect cells expressing a specific envelope receptor (i.e. TVA).

Symptoms of Exposure

Rabies presents initially as fever, body aches, and sore throat. Features of encephalopathy can develop from days to weeks after exposure, including anxiety, breathlessness, and hydrophobia due to laryngeal spasms while attempting to drink.

Modes of Transmission

The most common mode of transmission is through the bite of an infected animal, though it can occur through licking or scratching. There is documented exposures of infection through inhalation of aerosolized Rabies virus at high concentrations through mucous membranes. Exposure can also occur through injection (i.e. needle stick) and ingestion.

Host Range

All mammals are susceptible to rabies infection and can transmit the virus.

Environmental Stability

Enveloped viruses are rapidly inactivated when exposed to drying environments. However, it remains prudent practice in BSL-2 to disinfect with a broad-spectrum disinfectant, such as sodium hypochlorite (i.e. 1:10 bleach solution). This solution must be made daily.

Approvals

Experiments using rabies virus and rabies viral vectors require IBC approvals.

Test Methods for Replication Competent Virus

Not applicable

Laboratory Practices

Generally, rabies virus is classified as a **Biosafety Level 2** (BSL-2) organism. Rabies virus requires BSL2 practices and procedures for all work with the virus and Animal Biosafety Level - 2 practices and

procedures for all animal manipulations. Rabies viral vectors still require BSL-2 as they are still infectious.

1. No work with rabies 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 rabies 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 rabies 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.
8. Signs and labels must be placed to indicate each area where rabies 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 rabies virus must be conducted in a certified Class II biological safety cabinet.

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. Vaccination is only recommended once occupational health determines the risk of exposure. The vaccine is recommended for all individuals manipulating rabies virus in the laboratory as well as all individuals handling infected animals and contaminated cages/bedding.
2. Vaccination is not recommended for work involving the SAD B19 vaccine strain.
3. When the vaccine is recommended, it shall be administered before work commences and boosters shall be administered upon potential exposure.

Instructions in the Event of Employee Exposure

◆ EXPOSURE FROM SPLASH OR AEROSOLS – INHALATION

Report the incident to your supervisor and refer to the Emergency Action Plan for further instructions. The supervisor should submit an incident report through 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 Action Plan in the lab for further instructions. The supervisor should submit an incident report through 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 immediately after scrub. Seek medical attention at Occupational Medicine Services. After hours exposures should report to the closest Emergency Room. The supervisor should submit an incident report to document the event.

◆ EMERGENT EXPOSURES

For situations in which exposure to rabies virus occurred and medical treatment is an emergency, personnel should report to the Emergency Room, and ensure their supervisor completes incident report to document the event.

Decontamination

The most effective disinfectant against rabies 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 rabies virus, an Animal Biosafety Level - 2 (ABSL-2) area must be used and approved by the animal facility staff 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. 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. Depending on the size of the animal and procedures being performed, additional controls may need to be put into place for protection from aerosol.
5. The following information must be posted on the door of the animal room.
 - ◆ 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., rabies viral 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, contaminated cages and bedding

References

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

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): _____