FACT SHEET

Recombinant Rabies Viral Vectors

The following provides information on the use and containment of recombinant rabies viral vectors. Investigators should use these guidelines as part of their risk assessment when planning experiments with these vectors and preparing applications to the Institutional Biosafety Committee (IBC). Note the listed containment levels are the minimum that should be employed with these vectors: some experiments, such as the expression of toxins or oncogenes, may require higher levels of containment. The appropriateness of the containment should be considered as part of the investigator's risk assessment and will be reviewed by the IBC.

NIH Risk Group	RG2 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. Replication-deficient rabies vectors can be useful tools for investigation into neuronal trafficking or targeted expression in neurons. SADΔG-mCherry/EnvASADΔG is an example of a modified rabies virus. This modified version of the rabies virus forces neurons it infects to produce a red fluorescent protein called mCherry. mCherry makes the infected cells glow red so they are visible under a microscope. The benefit is the ability to trace a neural circuit on the cellular level as only connected/attached neurons are affected. Initial deletion: This modification deletes a gene which encodes the rabies virus envelope B19- glycoprotein (RG) and which is required for the production of competent or infectious viral particles from the virus genome 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 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. 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. Examples of this include EnvA, VSV-g, avian sarcoma leucosis virus glycoprotein, or HIV env. EnvA pseudotyped virus can only infect cells expressing the complementary receptor TVA. Since mammalian neurons do not express TVA, the injected virus cannot infect wild-type human neurons. If the virus is able to infect a TVA-positive neuron (for example, in transgenic mice), it can replicate and strongly label the first-order (initially infected) neurons, but since its genome lacks the B19 glycoprotein, it cannot infect other neurons by itself. In short, the risk for infection is specified by transgene expression and retrograde transport is limited to a single synapse. Thus the resultant virus becomes a "mono-synaptic" transneuronal tracer and significantly reduces the biohazardous risk because the virus has no potential to infect or trans-synaptically transport to any mammalian cells, including human and mice.
Biocontainement Level	BSL-2
Infectious to Humans/Animals	Yes

Route of Transmission	Percutaneous injury, such as animal bites. Potential non-bite modes of transmission include contamination of a pre-existing wound, contact of mucous membrane or respiratory tract with the saliva of an infected animal, exposure to aerosolised rabies virus in the laboratory (or from bats), or via organ transplantation from an infected donor, or inhalation of droplets
Laboratory Hazards	Accidental needlestick is a mode of transmission within research laboratories. Accidental ingestion of viral contaminated materials and inhalation are other routs of transmission. If working with infectious animal models, then bite wounds could transmit rabies virus infection.
Disease	Rabies virus can cause an acute infection, marked by progressive encephalomyelitis, and is usually fatal. The initial symptoms of rabies resemble those of other systemic viral infections, including fever, headache, malaise, and upper respiratory and gastrointestinal tract disorders. This prodromal phase typically lasts about 4 days, but can last as long as 10 days before specific symptoms develop.
Treatment/Prophylaxis	Consultation is available to determine if vaccination is appropriate for personnel working with recombinant rabies vectors. Vaccination is not needed for working with SAD B19 vaccine strain. Post-exposure rabies prophylaxis with vaccines together with the administration of rabies immunoglobulin (RIG) is highly effective but is a medical urgency. There is no established treatment for wild-type rabies once symptoms have begun, but supportive therapy may include intubation, sedation, mechanical ventilation, fluid and electrolyte management, and nutrition.
Replication Competent	Usually no but depends on pseudotyping and expression of envelope protein
RCV Testing	No effective methods for RCV testing
Disinfection	Effective disinfectants require a minimum of 20 minutes contact time. Use one of the following: • RECOMMENDED: Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach) • 5% Phenol • 70% Ethanol or Isopropanol
Animals	• ABSL-2. Animals must be injected in a Biological Safety Cabinet. Take precautions to avoid creating aerosols when emptying animal waste material. Soiled cages are disinfected prior to washing. Animal cages must be labeled with a biohazard sign. • If the B19 glycoprotein is not expressed in the recombinant vector and in the animals, stepdown to ABSL1 may be considered. The animals will be transferred to a clean cage, and the ABSL-2 cage will stay in the ABSL-2 quarantine space for appropriate waste disposal and cleaning. Once animals have been transferred to ABSL-1, they can be handled as with other ABSL-1 animals. The PI must provide a clear risk assessment for the experiments and the IBC will determine whether stepdown is appropriate and the time post injection for the stepdown to occur.

Sources:
http://web.stanford.edu/dept/EHS/prod/researchlab/bio/docs/Working_with_Viral_Vectors.pdf
http://www.dartmouth.edu/~ehs/biological/biosafety_docs/110_1_ibc_viral_vector_policy.pdf

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