Biosafety with PRV recombinants

Here are questions that we hear most often from institutional biosafety committees. Some biosafety committees have mistakenly assumed that the PRV strains used for transneuronal tracing are much like gene-delivery systems based on adeno-associated viruses or retrovirus vectors. It's helpful to clarify, early in the approval process, that PRV strains used for tracing are distinctly different.

1*. What is the virus/vector system you will be using?* The formal taxonomic name of pseudorabies virus (PRV) is Suid herpesvirus 1. It is classified as a member of the subfamily of alphaherpesvirinae of the family Herpesviridae. It is a CDC/NIH Class 2 agent. Attenuated versions of pseudorabies virus are used as transsynaptic tracing strains. Wild type virulent strains of PRV are not used as tracing viruses. The tracing strains are attenuated but are still replication competent. "Attenuation" means that their pathogenicity is markedly reduced compared to virulent PRV strains.

***2. List serotypes, if applicabl*e.** Not applicable. The strain names are: PRV-152, PRV-614, PRV-263, etc. Check the Table of available viruses on the website for specific information related to the virus you are requesting.

3. *List all plasmids used in generating the virus, what they encode, and where you obtained them.* In general, unlike AAV or retrovirus gene delivery agent, no plasmids are used to produce these viruses. They are self-replicating, autonomous viruses. Most PRV recombinants listed are derived from PRV Bartha, an attenuated live vaccine strain with reduced virulence. Each virus recombinant carries and expresses different markers (EGFP, RFP, B-galactosidase, etc.). Check the Table of available viruses on the website for specific information related to the virus you are requesting.

Papers describing the strain constructions:

Demmin GL, Clase AC, Randall JA, ***Enquist LW****, Banfield BW. Insertions in the gG gene of pseudorabies virus reduce expression of the upstream Us3 protein and inhibit cell-to-cell spread of virus infection. J. Virol., 75(22):10856-10869.*

Banfield, BW et al. Development of pseudorabies virus strains expressing red fluorescent proteins: new tools for multisynaptic labeling applications. J. Virol. 2003. 77, 10106–10112.

Kobiler O, Lipman Y, Therkelsen K, Daubechies I, and ***Enquist LW****. Herpesviruses carrying a Brainbow cassette reveal replication and expression of limited numbers of incoming genomes. Nat Commun. 2010;1: 146.*

Hogue IB, Bosse JB, ***Engel EA****, Scherer J, Hu JR, Del Rio T,* ***Enquist LW****. Fluorescent Protein Approaches in Alpha Herpesvirus Research. Viruses. 2015 7(11): 5933-61*

4. List the packaging cell line(s) used to generate the recombinant virus. Unlike AAV or retrovirus gene delivery agents that required a specific cell line to package virus particles, no plasmids or packaging cell lines are used to produce these viruses. They are self-replicating, autonomous viruses produced in standard tissue culture cell lines. The PRV recombinants can be grown on a variety of cell lines but the porcine kidney cell line, PK15, is commonly used.

5. Are you altering an existing virus for the purpose of studying that virus or are you using it as a vector? We are not altering these viruses. We are using them as replicating transneuronal tracers. We do not use these viruses as vectors.

6. What is the fraction of the viral genome contained in the recombinant DNA molecule?

<1/2 >1/2 but <2/3 >2/3

Not applicable. The DNA genome of PRV is approximately 142,000 base pairs of double stranded DNA. The reporter gene inserted in any one tracing strain is less than 1% of the viral genome.

7. Will you be increasing the replication capacity of the virus? NO

8. Does your vector system include a helper virus (e.g., retroviral and AAV systems, herpesvirus amplicons? NO

9. What genes are deleted from the backbone plasmid that will be packaged into your virus? Also indicate the viral genes that you will provide in trans to package your backbone plasmid. Not applicable. The viruses are not vectors; they are transneuronal tracers. No genes are provided in trans to package the viruses. They are self-replicating viruses.

10. What is the potential that replication competent virus will be produced during the in vitro generation of virus stocks? What is the potential that wild-type virus will be produced? Please provide a justification for your answers (also provide an estimate of the frequency of these events if possible). Not applicable. These viruses are live replicating, but attenuated, alpha herpesvirus strains.

11. Will you be using any other viruses in the same experiment (i.e., will cells, animals, or patients be co-infected with two or more viruses)? If so, please elaborate. NO

12. *Has the host range or tropism of the virus been modified in any way, genetically or biochemically? Specifically, did you or the company or collaborator you are receiving the virus from pseudotype this virus? If so, please describe.* No, the tropism has not been altered. These are not pseudotyped viruses.

RISK ASSESSMENT

Wild-type PRV. As stated above, the formal taxonomic name of pseudorabies virus (PRV) is Suid herpesvirus 1. It is classified as a member of the subfamily of Alphaherpesvirinae of the family Herpesviridae. It is a CDC/NIH Class 2 agent and when we work with samples of virus larger than a few milliliters, we work with it under Biosafety Level 2 conditions. The standards for BSL2 relative to PRV work have been reviewed by Card and Enquist (1999), Strick and Card (1992) and Card et al. (1990). PRV is not known to cause human infections in the laboratory. However, caution when using PRV is appropriate because there are reports of rare human infection in farm workers.

Diseases caused by wild-type PRV. Wild-type PRV causes a natural, economically important disease (Aujeszky's disease) in swine. It also causes the same disease in other members of the Suidae family. PRV causes fatal "mad itch" in cattle, dogs, and some wild animals.

Wild-type PRV has a broad host range, infecting many classes of mammals and avian embryos, but horses, reptiles, insects and some higher primates, including humans, are mostly resistant. The pathogenesis and molecular biology of the virus have been studied in some detail. PRV is not known to cause human infections in the laboratory. **There are no documented cases of accidental lab infections of humans.** Even so, caution when using PRV is appropriate because of reports of rare human infection in farm workers in China. Among laboratory animals, the rabbit is the most sensitive to

infection. Rodents are also highly susceptible. PRV appears to infect some laboratory primates, but not others. Its usefulness as a transneuronal tracer following injections into the central nervous system or into the periphery in nonhuman primates has not been fully explored.

Laboratory infections. In general, infection of laboratory animals can happen only by injection, scarification or biting, and not by aerosol or inadvertent or casual contact. We have infected animal cages adjacent to uninfected animal cages and have never observed any cross infection. On occasion, a student inadvertently has left an uninfected animal in a cage with an infected animal and no cross infection was noted.

Using the attenuated strains of PRV under the conditions used for BSL2, PRV presents no danger to pet dogs, cats, and rodents. However, dogs and other pets should not be allowed in lab areas or lab animal quarters. For pets to be infected, they would need to be directly injected with virus or eat an infected mouse or rodent carcass. As noted above, PRV is not spread in the lab by aerosols, and it is not secreted in infected animal feces or urine.

When small volumes of virus are used, as they are in microscope or electrophysiology experiments, the probability of inadvertent infection of nearby susceptible animals is vanishingly small. Certainly, the probability of infecting humans approaches zero.

Tracing strains of PRV are attenuated vaccine strains. For most neural tracing studies with PRV, attenuated vaccine strains based on PRV Bartha are used. PRV Bartha was isolated in the 1960s and was considerably less virulent. It was the basis for a live PRV vaccine. These strains induced mild to no symptoms in infected animals despite replicating and spreading in neural circuits. Infected animals live at least twice as long with essentially no symptoms as those infected with wild-type virus. Wild-type PRV strains are rarely used for tracing because they are too virulent and kill animals before there is extensive spread in the nervous system. Attenuated viruses may require more virions to initiate an infection and cause milder disease compared to wild-type virus.

PRV Bartha-based live attenuated vaccines are used widely in the swine industry. Bartha is an attenuated PRV vaccine strain, derived from a field strain that was isolated in Hungary and that was attenuated via multiple passages on cultured chicken cells and embryos, resulting in several mutations. The most striking feature in the Bartha genome is a 3-kb deletion in the US region, resulting in the partial loss of US2, most of US7 (gI), and a complete deletion of US8 (gE) and US9. The Bartha genome is completely sequenced as well as compared to the genome of two other virulent PRV strains. The Bartha vaccine and its derivatives have been highly successful and are well-characterized.

Literature:

Bartha. A. Experimental reduction of virulence of Aujesky's Disease virus. Magy Allatory, Lapja. 1961 16:42-45.

Pomeranz LE, Reynolds AE, Hengartner CJ. Molecular biology of pseudorabies virus: impact on neurovirology and veterinary medicine. Microbiol Mol Biol Rev. 2005 Sep;69(3):462-500. PMCID: PMC1197806

McFerran, JB, and Dow, C. Studies on immunization of pigs with the Bartha strain of Aujesky's disease virus. Res. Vet. Sci. 1975. 19:17-22

Mengeling, WL, Lager, KM, Volz, DM and Brockmeier, SL. Effect of various vaccination procedures on shedding, latency, and reactivation of attenuated and virulent pseudorabies virus in swine. Am. J. Vet. Res. 1992. 53:21i64-2173.

Complete genome sequence of wild-type PRV and PRV Bartha. Literature:

*Szpara, JL, Tafuri, YR, Parsons, L, Shamim, SR, Verstrepen, KJ, Legendre, M, and **Enquist, LW**. A wide extent of interstrain diversity in virulent and vaccine strains of alphaherpesivruses. 2011. PLoS Pathogens &: e1002282*

PRV Bartha is markedly less virulent in rodents than the virulent strain. Mice infected with virulent (e.g., PRV Becker, PRV Kaplan, or PRV-NIA3) strains self-mutilate their flank skin in response to virally induced pruritus, die rapidly with no identifiable symptoms of central nervous system (CNS) infection such as behavioral abnormalities, and have little infectious virus or viral antigen in the brain. In distinct contrast, animals infected with an attenuated PRV vaccine strain (PRV Bartha) survive approximately three times longer than wild-type PRV-infected animals and have an abundance of infectious virus in the brain at the time of death. Interestingly, these animals have no skin lesions and do not appear pruritic at any time during infection. The severe pruritus and relatively earlier time until death induced by wild-type PRV infection may reflect the peripheral nervous system (PNS) and immune responses to infection rather than a fatal, virally induced CNS pathology. Based on previously characterized afferent (sensory) and efferent (motor) neuronal pathways that innervate the skin, we deduced that wild-type virulent strains transit through the PNS via both afferent and efferent routes, whereas PRV-Bartha travels by only efferent routes in the PNS en route to the brain.

Literature:

*Brittle EE, Reynolds AE, **Enquist LW**. Two modes of pseudorabies virus neuroinvasion and lethality in mice. J. Virol., 2004. 78(23):12951-12963.*

*Laval K, Vernejoul JB, Van Cleemput J, Koyuncu OO, **Enquist LW**. Virulent PRV infection induces a specific and lethal systemic inflammatory response in mice. J Virol. 2018. 92(24): e01614-18.*