



Stanford University
Department of Comparative Medicine – Veterinary Service Center

This VSC Guideline has been written in order to clearly communicate current **recommendations** to facility users. Questions should be directed to Dr. Claude Nagamine (cnagamin@stanford.edu, 650-498-4773).

Guidelines for the Use of Biological Materials in Rodents

Biological materials that are of primary concern to A-PLAC and VSC include (but are not limited to) any cell line (rodent or non-rodent) previously passed in rodents or grown on murine feeder cells obtained from rodents outside of your colony; transplantable tumors passed in rodents; biologics purified from tumors grown in rodents; any rodent tissue, rodent antibody preparations, rodent stem cells, rodent body fluids or sera obtained from rodents outside of your colony, virus stocks, and media from rodent cell cultures. These biological materials may harbor adventitious pathogens. When these biological materials are introduced into rodents as part of an experimental procedure the pathogens are capable of infecting and propagating in the mouse, rat, or hamster host, and, in some cases, are capable of being transmitted to humans, e.g., lymphocytic choriomeningitis (LCMV). The health of the Stanford rodent colonies and the VSC personnel, and the research of other investigators can be endangered by inadvertent introduction of pathogens when untested biological materials are inoculated into rodents. Therefore it is the **recommendation** of the VSC that all biological materials to be inoculated into rodents be tested by PCR for adventitious viral pathogens and *Mycoplasma pulmonis* prior to the start of these experiments unless the rodents are to be quarantined in a biohazard suite.

- Although prevalence have decreased in recent years, 25% of 297 mouse, rat, hamster, and human transplantable tumors and 69% of 465 murine leukemias and tumors have historically been found to be contaminated with mouse or rat pathogens in the past (Lab Anim Sci. 1993. 43:296).
- Lactate dehydrogenase elevating virus (LDEV) was recently detected in Becton, Dickinson and Company's Matrigel™ (BD Newsletter, March 21, 2007). Matrigel™ is purified from Engelbreth-Holm-Swarm tumors passaged in mice. Immunocompromised or susceptible mouse strains inoculated with xenografts grown on contaminated Matrigel™ can develop polioencephalomyelitis.
- Mousepox (ectromelia virus) outbreaks, which have historically decimated research colonies at NIH, NMRI, and several research institutions, have been traced to commercially available mouse serum (Comp Med. 2009. 59:180). Lots of known infected sera have never been fully accounted for.
- LCMV transmitted from hamsters or nude mice inoculated with LCMV-infected tumor cell lines (JAMA. 1992. 267:1349) or *Toxoplasma gondii* stocks (Inf Imm. 1985. 50:917) have been implicated in the infection of caretakers and researchers. Prenatal LCMV infection is neuroteratogenic to the fetus (Ann Neurol. 2007. 62:347). Postnatal infections result in symptoms ranging from mild flu-like disease to aseptic meningitis and, in severe cases, death.

What should be tested:

1. Any biological material not obtained by primary isolation from rodents currently housed in your colony at Stanford University should be tested unless there is written documentation that the biological material is free from murine pathogens. Written documentation should include: date of testing, list of screened pathogens, diagnostic test(s) performed, and the laboratory in which the test was performed. Biological materials harvested from colonies under quarantine should also be tested unless they will be used in rodents within the same room.

2. Human-origin or non-rodent tumors and cell lines should be tested unless credible documentation is available that they have never been passaged through or exposed to rodents or grown on rodent feeder cells.
3. Cells from culture collections, e.g., American Type Culture Collection (ATCC), should be tested unless written documentation exists to show that they have been tested. ATCC does not test cell lines for contaminating murine pathogens.
4. Virus and protozoa stocks that will be inoculated into rodents if the rodents will be subsequently housed in a regular animal room. This includes viral vectors (lentivirus, AAV, adenovirus, MMTV, etc.) that are amplified using cell lines and protozoal parasites that were isolated from or were passaged in rodents outside of your colony. It is appropriate and probably more efficacious to test the cell line(s) used to amplify your virus or parasite stocks if the same cell line is used to generate different virus or parasite stocks.

Test methodology: Biological materials are tested by PCR. The recommended panel for biological materials to be introduced into **mice** is currently RADIL's IMPACT Profile-I. This assay tests for the pathogens routinely screened by the VSC mouse sentinel program.

If the biological materials are to be introduced into rats or hamsters or if you wish to use a different diagnostic laboratory or testing panel, please contact Dr. Claude Nagamine (cnagamin@stanford.edu, 650-498-4773) to avoid the cost of inadequate testing and the need for a repeat test.

One vial of 10×10^6 cells or 1 vial of 0.5 ml/vial of serum, ascites, virus stocks, or other liquid are typically required. If multiple cell lines are involved, you can pool up to 5 cell lines per sample (2×10^6 cells/line; total = 10×10^6 cells) to save on cost. Note that if the pooled sample is found to be positive for a pathogen, you **MUST** test each cell line for that specific pathogen to determine if one or all cell lines are contaminated. In addition, if the biological material is contaminated with *Mycoplasma* sp., the *Mycoplasma* must be speciated to rule out the murine pathogen *M. pulmonis*.

If you wish to ship the samples yourself, be aware of the regulations, training, and certifications required for the shipping of biological materials on dry ice as outlined on Stanford's EHS website ("Shipping of Hazardous Materials"), as well as the website of the diagnostic laboratory, e.g., www.radil.missouri.edu.

If you wish for the VSC Diagnostic Laboratory to ship the biological materials, please contact the VSC Diagnostic Laboratory (650-735-8305) for specific instructions and the charges for shipping and handling.

NOTE: A pdf copy of the results MUST be sent to Dr. Claude Nagamine (cnagamin@stanford.edu) for documentation that the biological materials have been adequately tested. The results will be reviewed and attached to your A-PLAC protocol.