Stanford University  
Department of Comparative Medicine – Veterinary Service Center

This VSC Guideline is intended to communicate current recommendations to animal 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) cell lines (rodent or non-rodent) previously passed in rodents or grown on feeder cells obtained from rodents outside of your colony; transplantable tumors or biologics purified from tumors passed in rodents; any rodent tissue, antibody, stem cell, body fluid or sera obtained from rodents outside of your colony; virus or parasite stocks produced from rodent or non-rodent cell cultures or passed in rodents; and serum components preabsorbed with rodent cells. These biological materials may harbor rodent pathogens and when introduced into the mouse, rat, or hamster, the pathogens may propagate and spread in the animal facility. In some cases, the pathogen is zoonotic and may be transmitted to humans, e.g., lymphocytic choriomeningitis (LCMV). The Stanford rodent colonies, the health of Stanford personnel, and the research of Stanford investigators are endangered 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 to be tested for pathogens. The only exception is if the rodents are to be quarantined in a biohazard suite in which no rodent in the biohazard suite is ever returned to the main vivarium.

• Although prevalence have decreased in recent years, 25% of 297 mouse, rat, hamster, and human transplantable tumors and 69% of 465 murine (i.e., mouse or rat) leukemias and tumors were found to be contaminated with mouse or rat pathogens in the past (Lab Anim Sci. 1993. 43:296).

• Lactate dehydrogenase elevating virus (LDEV) was detected in Matrigel™ (BD Newsletter, March 21, 2007). Matrigel™ is purified from Engelbreth-Holm-Swarm tumors passaged in mice. Immuno-compromised or susceptible mouse strains inoculated with xenografts grown on contaminated Matrigel™ can develop polioencephalomyelitis. At Stanford, LDEV was identified in tumor cell lines in 2011 and two different parasite stocks in 2012.

• Mousepox (ectromelia virus), which have decimated mouse colonies at NIH, NMRI, and several research institutions in the past, was identified in commercially available mouse serum (Comp Med. 2009. 59:180). Lots of known infected sera have never been fully accounted for.

• Hamsters and nude mice inoculated with LCMV-infected tumors have infected caretakers and researchers (JAMA. 1992. 267:1349). Toxoplasma gondii stocks have also been found to be contaminated with LCMV (Inf Imm. 1985. 50:917). LCMV infection is neuroteratogenic to the human fetus (Ann Neurol. 2007. 62:347). Human symptoms range from flu-like disease to aseptic meningitis and, in severe cases, death.

• Rabbit complement H2, preabsorbed with pooled mouse spleen and red blood cells, obtained from a vendor was found contaminated with Minute Virus of Mice (MVM) and LCMV (L. Zitzow. 2012 ACLAM Forum. Personal communication).

What should be tested:

1. Any biological material not obtained by primary isolation from rodents currently housed in your colony at Stanford should be tested unless there is written documentation that the biological material is free from...
murine pathogens. Documentation should include: testing date, list of screened pathogens, diagnostic test(s) performed, and the laboratory that performed the test. 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, cell lines, or serum components should be tested unless credible documentation is available that they have never been passaged through or established in rodents, grown on rodent feeder cells, or for serum or serum components, preabsorbed with rodent cells.

3. Cells, parasites, and viruses 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 for contaminating mouse or rat pathogens.

4. Virus and protozoa stocks, irrespective of BSL status, 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 produced using cell lines and protozoal parasites that were isolated from or passaged in rodents outside of your colony. It is appropriate to test the cell line(s) used to produce 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. Please contact Dr. Claude Nagamine to determine if the cell line was previously tested. If the cell line was previously tested and you obtain the cell line from a reputable repository, e.g., ATCC, the cell line will be exempt from testing. The VSC Diagnostic lab has contracted with Charles River Laboratories (CRL) to provide mouse, rat, and combined mouse and rat panels that screen for the most common pathogens. Turnaround time is typically 10 - 14 days.

If the biological materials are to be introduced into a rodent species other than the mouse or rat or if you wish to use another diagnostic lab please consult with Dr. Claude Nagamine (cnagamin@stanford.edu, 650-498-4773) to avoid the risk of inadequate testing and the need to repeat the test.

Samples to submit to the VSC Diagnostic lab (1 of the following):

- (1) cryovial of 0.2 - 0.5 ml of undiluted serum, ascites, virus, or parasite stock, or other liquid.

- (1) cryovial of 2 - 10 x 10^6 cells in a volume of ≤1 ml. Do not pellet the cells. Do not exceed 10 x 10^6 cells (less is OK). Leave the cells in the original tissue culture medium.

- (1) cryovial of a pool of up to 5 cell lines in a volume of ≤1 ml. 2 x 10^6 cells/ cell line. Do not pellet the cells. Do not exceed a total of 10 x 10^6 cells (less is OK).

Note that if a pooled sample is found to be positive for a pathogen, you should test each component for that specific pathogen to determine which component(s) is/are contaminated.

If you wish to ship the samples yourself to CRL, be aware of the regulations for the shipping of dry ice as outlined on Stanford’s EHS website (“Shipping of Hazardous Materials”), as well as the website of the diagnostic laboratory.

NOTE: If you ship the samples yourself, a copy of the results must be sent to Dr. Claude Nagamine (cnagamin@stanford.edu) to document that the biological materials have been adequately tested.