Screening Tumor Cell lines and Biological Materials for Infectious Microbes:
Mouse/Rat Antibody Production (MAP/RAP) TEST
Molecular Testing of Biological Materials-Mouse/Rat (MTBM-M/R)

Sample Preparation and FAQs:

Protecting the health status of experimental animals from infectious disease is critical for obtaining valid, reliable *in vivo* data. An important potential source of viral infections that are introduced into an animal facility is contaminated biological materials that are injected into live animals. Biological materials include tumor cell lines, stem cells, serum, antibodies, and other materials which may have been produced in the presence of serum from infected mice or rats or become contaminated during processing in the laboratory. Contamination poses a risk to multiple populations in an animal facility, and in the case of zoonotic agents such as Lymphocytic Choriomeningitis Virus, there can be health risks for research staff.

The Mouse/Rat Antibody Production (MAP/RAP) test has been the method of choice for screening biological materials, but considerable disadvantages include the time delay to get results, six to eight weeks, and the cost. **The University of Missouri Research Animal Diagnostic Laboratory (RADIL) and several other outside testing laboratories have now validated the sensitivity of an alternative molecular-based assay.** Comparison of the molecular based assay with the traditional MAP/RAP test suggests that for detection of representative mouse DNA and RNA viruses in tissue culture samples, the PCR technique had equal or greater sensitivity and not only costs less to run than the MAP test but results are obtained within two weeks of sample submission.

Beginning in April 2006, the LASP recommends use of the Molecular Testing of Biological Materials-Mouse/Rat (MTBM-M/R) Test as the screening test of choice for the majority of samples. For certain specimens however, (ex. primary cells, tissues, tumors) MAP/RAP-testing may be more accurate, and the decision to test by MAP/RAP or MTBM-M/R will be made by the Animal Health Diagnostic Lab (AHDL) following review of sample submission information.

Projects involving tumor cell lines and potentially contaminated biological materials may not commence until the ACUC has received documentation of negative test results – either MAP/RAP Tests or MTBM-M/R Tests.

Questions and Answers

What needs to be tested?
All tissue/cell lines, tumors, stem cells, and biological products originating off the NCI-Frederick campus, including tissues/cell lines from the NIH campus, commercial suppliers such as the ATCC (American Type Tissue Collection) and all animal serum products must be tested.

How are samples prepared for submission?

MTBM-M/R Testing:
Samples will be submitted to the LASP Animal Health Diagnostic Laboratory (AHDL) as in the past, but the AHDL will submit samples to RADIL each Wednesday. Samples received on Tuesday pm, Wednesday, Thursday, or Friday will be sent the following week. **For testing cultured cells, two small cryovials of each sample with 1 x 10^7 cells per vial. Cells may be in the form of pellet or in growth medium of PBS. For testing non-cellular material, such as material extracted from cell culture, submit two cryovials with 0.5 ml per sample. Samples not prepared in this manner will automatically be tested by the MAP/RAP Test.**

MAP/RAP Testing:

Cell/Tissue Cultures:
At least six (6) ml of cells and spent media should be submitted. There should be at least four (4) to six (6) million cells per ml.

An easy method of doing this is to:

Adherent lines: use a fully sheeted "T" (75 sq. cm) flask, remove all but 6-8 ml of the spent media and either scrape, trypsinize or freeze the cells into the remaining 6-8 ml of spent media and decant the cells and spent media into a 15ml freezable centrifuge tube. The tube should be frozen at –70oC until submission to the AHDL.

Non-Adherent lines: use a turbid (to the naked eye) "T" (75 sq. cm.) flask; pour approximately 40 ml of the cell suspension into a 50ml centrifuge tube. At moderate speed, centrifuge the contents of the tube, when completed; decant approximately 30ml of the supernatant. Submit the remaining 8-10 ml of spent media with the cell pellet. The tube should be frozen at –70oC until submission to the AHDL.

Tumors/Tissues:
At least six (6) ml of a 10% suspension by weight should be prepared and ground in a tissue grinder. Sterile cell culture media or sterile normal saline should be used as the diluent. The suspension may be frozen at -70 C until submission to the AHDL.

Monoclonal Antibodies and other Non-Cellular Biologicals:
At leas six (6) ml of the non-cellular biological material should be submitted. The concentration should be, whenever possible, the same concentration intended for *in vivo* studies. The more concentrated the material, the greater the sensitivity of the MAP/RAP Test.

Where can I get the necessary paperwork?
An on-line submission form is available at the AHDL web site:
http://web.ncifcrf.gov/rtp/lasp/intra/ahdl/Map_Doc.asp
The form must be filled out completely. Questions or concerns regarding the on-line form can be addressed to the AHDL at 301-846-1134.

Where should samples be taken?
Samples may be given to LASP Building Manager or taken directly to Building 429-AHDL, Monday through Friday 8am until 4:00pm.

Which agents will be evaluated?

MAP Test
Mouse hepatitus virus (MHV)
Polyoma virus (POLY)
Sendai virus (SEN)
Pneumonia virus of mice (PVM)
Reovirus 3 (REO3)
Minute virus of mice (MVM)
Theiler's murine encephalomyelitis virus (GDVII or TMEV)
Lymphocytic coriomeningitis virus (LCMV)
Ectromelia virus (ECT)
Lactic dehydrogenase-elevating virus (LDHV)
Hantaan virus (HAN)

MTBM-M
Mouse hepatitus virus (MHV)
Polyoma virus (POLY)
Sendai virus (SEN)
Pneumonia virus of mice (PVM)
Reovirus 3 (REO3)
Minute virus of mice (MVM)
Theiler's murine encephalomyelitis virus (GDVII or TMEV)
Lymphocytic coriomeningitis virus (LCMV)
Ectromelia virus (ECT)
Lactic dehydrogenase-elevating virus (LDHV)

Mycoplasma spp. (MYCO)
Mouse parvovirus (MPV)
Mouse norovirus (MNV)
Mouse rotavirus (EDIM or MROTA)
Mouse adenovirus (MAD)
Mouse cytomegalovirus (MCMV)

RAP Test
Rat coronavirus/Sialodacryoadenitis virus (RCV/SDAV))

Polyoma virus (POLY)
Sendai virus (SEN)
Pneumonia virus of mice (PVM)
Reovirus 3 (REO3)
Kilham virus/Toolan's H-1 virus (KRV/H-1)

Hantaan virus (HAN)

MTBM-R
Rat coronavirus (RCV)
Sialodacryoadenitis virus (SDAV)

Sendai virus (SEN)
Pneumonia virus of mice (PVM)
Reovirus 3 (REO3)
Kilham virus (KRV)
Toolan's H-1 virus (H-1)

Mycoplasma spp. (MYCO)
Rat parvovirus (RPV)
Rat murine virus (RMV)
Lymphocytic coriomeningitis virus (LCMV)
Rat cytomegalovirus (RCMV)
Seoul virus (SEO)
Mouse adenovirus (MAD)
Theiler's murine encephalomyelitis-like virus (RTMEV)

Who pays for this testing?
For investigators with Center for Cancer Research (CCR): Testing of all cell lines to be used in NCI facilities in Frederick or Bethesda will be centrally funded.

All other investigators (NCI-Non-CCR, other NIH Institutes or non-NIH Agencies) must pay for each line tested and must have an NCI-Frederick Cost Center Number. Investigators may contact their Administrative Office for such information. Requests for pricing can be directed to Wayne L. Christensen 301-846-1567 wchistensen@mail.ncifcrf.gov or Peter L. Gorelick 301-846-1134 gorelickp@mail.ncifcrf.gov

Are there any exceptions to the testing requirements?
Spontaneous tumors that arise within NCI-Frederick animals, provided that the cells are not collected during the time of a virus outbreak, may be exempted from testing; however, when a disease outbreak is confirmed within an NCI or NIH animal facility, all biological materials passaged *in viv*o within the previous six weeks should be re-submitted for testing or discarded so that they are not re-introduced into animals. If the passaging history or the parental origin is uncertain, the sample should be resubmitted and tested for the listed agents.

What about fresh human tissue? Yes, although rare, agents such Lymphocytic Choriomeningitis Virus (LCMV) and Reovirus may be transmitted from humans to animals. These tissues need to be from documented HIV-negative and Hepatitis B negative patients.

How soon will results be returned?
MAP/RAP Testing: 5-6 weeks
MTBM-M/R Testing: 10 days to 2 weeks

What do I do with the results?
For Bethesda:
Fax results to the Bethesda ACUC Coordinator at 301-402-1276 and specify to which ASP they should be attached.

For Frederick:
Attach results to your ASP or if the ASP was already submitted, fax results to Frederick ACUC Coordinator Michelle M. Gottholm Ahalt at 301-846-6590 and specify to which ASP they should be attached.

There is no time limit on validity of results; however, investigators are encouraged to update testing periodically, such as every 10 years, as the sensitivity and agents screened are likely to increase over time.