

Guideline for Biological Material Risk Factors and Testing Requirements

Animal studies are an integral component of research at the NCI at Frederick. The overall health status of the animals utilized in research plays a crucial role in the validity of experimental results generated from in vivo studies. Health risks may come from a variety of sources, such as the introduction of infected rodents into the animal facility, the presence of feral animals, and experimental and genetic manipulations. The Laboratory Animal Sciences Program (LASP) makes great efforts in preventing the introduction of infectious agents through an exhaustive Receiving & Quarantine Program, extensive health monitoring and in cooperation with Facilities, Maintenance, and Engineering (FME), a pest control program. One important and potentially devastating source of adventitious viral infections is through the injection or implantation of various biological materials. Historically, many of these biological materials included tumor and cell lines, but now may include materials such as monoclonal antibodies, antigens, and non-cellular materials produced in the presence of rodent sera. Although an investigator may be diligent in having biological material tested for infectious agents through MTBM (Molecular Testing of Biological Materials) testing [viral screening], there exists a threat of cross contamination from other biological materials which are manipulated in the same laboratory space and/or containment devices.

As a result, the NCI at Frederick ACUC will only accept MTBM testing results. Such results must include a screen for agents listed in Appendixes A or B. A copy of these results must be appended to your applicable Animal Study Proposal submission. Please refer to the AHDL website [<https://ncifrederick.cancer.gov/lasp/ahdl/Default.aspx>] for additional information regarding the available testing modalities and guidance for requesting testing services in advance of proposing biological materials for use in NCI at Frederick animal facilities.

1. Any biological material to be injected into live animals must be MTBM tested prior to its use in animals. A copy of the test results must be provided to the ACUC office, where it will be appended to the relevant ACUC protocol. Material testing positive for specific pathogens may be excluded from use in animals, depending upon the status of the facility in which the intended recipient animals are kept. Duplicate vials of the pathogen-free (or of known specific pathogen status) material can be stored frozen and subsequently injected into animals without the need for additional MTBM testing if the material was simply thawed for the injection. If the material was obtained from a vendor and the manufacturer's lot was MTBM tested, material from the SAME lot may be injected without the need for additional testing provided that a copy of the test results was provided to the ACUC office.
2. Cells or other biological materials previously MTBM tested may be subsequently manipulated in culture (transfection by viral or plasmid vector, sub-cloned, expanded for injections in culture, etc.) and used in animals without the need for additional testing, provided that the samples did not come into contact with untested rodent material or rodent-derived products at any time during the manipulation, and that the cells have not been in continuous culture for more than 3 years. Otherwise, the biologic material must be once again MTBM tested prior to its use in animals. Cell culture facilities or laboratories performing manipulations on biological materials to be injected into animals without additional MTBM testing will be screened for cleanliness and compliance with this policy

during the protocol review, and by post-approval monitoring performed by individuals appointed by the ACUC for such purposes.

Here are some important factors to consider:

- All biological materials pose a potential and significant health risk to animals. This includes material from humans, which may be contaminated with zoonotic agents such as Lymphocytic Choriomeningitis Virus (LCMV).
- Certain synthetic antigens and compounds may be exempted from testing by the Frederick-ACUC on a case-by-case basis.
- MTBM testing performed by different vendors may vary, and some vendors may not provide adequate testing or test results to permit ACUC acceptance of the MTBM test results. Investigators should consult with the ACUC office for a listing of approved vendors and test results.
- There is no prohibition preventing investigators from receiving virally infected materials for in vitro uses only. However, it is important to consider that cross contamination can occur within biological safety cabinets or other approved engineering controls (i.e. vented hoods). Therefore, a hood should be thoroughly decontaminated before it is used to work with material intended for in vivo use.
- Spontaneous tumors, which develop in animals at NCI at Frederick, are exempted from viral testing, providing they are not collected during a health outbreak and are used in a facility with equivalent health status.
- If a health outbreak is detected and confirmed within a NCI at Frederick animal facility, all biological materials passaged in vivo within a six-week period or longer depending on the agent must be viral screened.

Requires Biological Testing

The following materials require testing prior to use in NCI at Frederick animal facilities. Testing must be species-appropriate (testing for mouse or rat pathogens for material to be placed into mice or rats, respectively). Copies of the MTBM test results must be appended to the applicable ASP form and/or modification before ACUC approval will be granted. This includes but is not limited to the following:

- Rodent Tumors
- Rodent Cell Lines
- Manipulated Rodent Tumor Cell Lines
- Rodent Derived Antibodies
- Human Cell Lines [unless proven that there is no risk of exposure to rodent products] *
- Naturally obtained Cytokines [purified not generated by recombinant technology]
- Rodent By-Products [e.g., sera]
- Natural Materials [e.g., virus stocks grown in or containing rodent products]
- Other biological materials containing murine derived products
- Cryopreserved rodent tumors derived at the NCI at Frederick

- Matrigel [lot specific testing required], Basement Membrane Extract (BME), other rodent-derived growth supports, including those used for *in vitro* growth

Requires Vendor Specification Sheets

Copies of the vendor specification sheets and proper plan to implant into mice/rats must be provided to ensure that no rodent materials were used in the preparation of the material or that the purification process [by the vendor or in the laboratory] ensures complete destruction or elimination of any viral contaminants. Copies must be appended to the applicable ASP form and/or modification before ACUC approval will be granted.

- Purified Antibodies
- Antigens
- Proteins
- Cell extracts

Do NOT Require AHDL Biological Testing

- Fresh spontaneous or induced rodent tumors and/or cell lines derived from rodents within the NCI at Frederick animal facilities and used within the same animal facility or a facility of equal health status [facility manager pre-approval required]
- Human Primary Tumors *
- Materials of non-rodent origin [i.e., rabbit] Note: these materials will require MTBM testing if they came into contact with rodent-derived materials during formulation
- Synthetic materials
- Bacterial growth media not containing any rodent products

NOTE: There is no time limit on the validity of results. However, investigators are encouraged to update testing periodically, such as every ten years, as the assay sensitivity and agents screened are likely to increase over time. Tests prior to 2006 were conducted with less-sensitive, non-PCR methods which are no longer valid thus the material requires repeat MTBM testing before the material can be injected.

If a cell line has been previously tested by an NCI Principal Investigator and is shared with another NCI PI, retesting is not required unless the cell line has been subsequently manipulated and exposed to rodent material. However, a copy of the original MTBM testing results must be forwarded to the ACUC office for inclusion in the recipient Investigator's Animal Study Protocol file.

If, for any reason, Biological Testing cannot be performed prior to study initiation, the experiment may be performed in the Receiving and Quarantine facility or other ACUC/ LASP approved quarantine facility. Please contact the Receiving and Quarantine manager for space/staff availability. Failure to provide MTBM testing results to the ACUC office prior to use of the biological material in animals housed outside of the Receiving and Quarantine facility is a Protocol violation.

- * Please refer to the *ACUC Guidelines Regarding Engraftment of Human Cells or Tissues into Immunodeficient Mice* for guidance on human pathogen testing and housing requirements for using human materials.

Appendix A

MTBM – Mouse Pathogen List

Mouse hepatitis 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 choriomeningitis 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)

Appendix B

MTBM – Rat Pathogen List

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)

Retheliovirus (k)

Mycoplasma spp. (MYCO)

Rat parvovirus (RPV)

Rat murine virus (RMV)

Lymphocytic coriomeningitis virus (LCMV)

Rat cytomegalovirus (RCMV)

Seoul virus (SEO)

Mouse adenovirus (MAD)