TITLE: Biological Materials Testing

Background

Biological materials such as transplantable tumours, hybridomas, cell lines, blood and other biological products may harbor adventitious pathogens. When introduced into research animals, these agents may cause disease and/or interfere with research results. Rapid and effective assays are available to monitor microbiologic contamination of such material. Any product of animal origin or material that has been passaged through animals should be tested prior to being used. Even if obtained from an impeccably clean source, testing is still crucial, as the material may have become contaminated at the time of collection, by co-housing in liquid nitrogen or through handling or processing. Biological materials used in research animals often involve the use of cryopreserved mouse (murine) cell lines, for example. It’s important to recognize that cell lines obtained from commercial repositories (eg. ATCC) typically have NOT been rigorously tested for the presence of murine pathogens.

Introduction of these untested materials into the colonies at the ANU may cause subclinical disease that may alter research results and/or compromise the health status of not only the injected mice, but the colony.

Procedure

The Australian National University Animal Ethics Committee now requires biological materials to be tested for the pathogens listed below before they are used on animals or transferred into animal holding facilities.

A. Principal Investigators (PIs) are responsible for testing all cell lines and biological materials BEFORE their administration into any rodent. Mandatory testing or retesting is required every five (5) years. Frequency of testing also depends on the origin of material and previous pathogen testing.
B. When any pathogen is diagnosed as a result of sentinel surveillance, biological materials used in the rodents in the affected room must be tested for that pathogen. Faeces will be routinely collected and stored from animals injected with biologics, for further investigation if necessary.

C. The Biological Agent Form must be completed, samples submitted through animal.shipments@anu.edu.au and results evaluated by the ABRF Veterinarian before administration of the biological material.

D. Principal Investigators (PIs) currently using biological materials in their animals or, anticipating administration of biological materials, must list the biological materials in their protocol to the AEEC.

E. Biological Materials that May Require Testing:
Any biological material that has originated from rodents or which have been exposed to rodents directly (in vivo passage) or indirectly (e.g. via tissue culture media additives).
This includes cell lines, viruses, tissues, rodent serum, bone marrow, myelomas, hybridomas, spermatozoa, ascites fluid (not if harvested; only if being passaged into new host), purified proteins including antibodies, transplantable tumours etc.

Exemptions
Viruses or viral vectors which have been synthesized without any exposure to murine cell lines or tissues.
Rodent fresh cells or tissues harvested from within an SPF ANU rodent colony, do not require pathogen testing.
Primary human cells lines or tissues are also exempt from the murine panel, but should be tested for Mycoplasma.
If obtaining antibodies etc. from commercial sources, please request lots that have had rodent pathogen testing, if available, and forward a copy of the results to animal.shipments@anu.edu.au.

Please contact the ABRF Veterinarian Dr Sonya Glasson on 61257755 sonya.glasson@anu.edu.au to discuss testing prior to using biological materials in animals.

Testing required for studies involving mice:

1. Mycoplasma
2. Murine Parvoviruses (PVM, MVM)
3. MCMV
4. MHV
5. EDIM (Rotavirus)
6. TMEV
7. LCMV
8. REO-3
9. LDEV

Cost

Testing for these 9 agents at a commercially pathogen laboratory costs $313.50 + shipping. If sufficient time is allowed, then up to 4 samples can be pooled together, and bulk shipments sent, reducing costs to ~$85 per sample.
1. Sample Preparation

Samples should be representative aliquots of the larger (master cell bank) volume of material proposed to be utilized in the project. Collection of material for PCR pathogen testing should be performed ASEPTICALLY to prevent inadvertent contamination of the samples.

2. Cell Cultures

Two (duplicate) vials of ideally $> 1 \times 10^6$ cells, spun down at approx 1200 rpm for 2 minutes and supernatant removed and the cell pellet frozen and shipped on dry ice.

If possible, samples should be passaged without antibiotics prior to submission. (or have a minimum of two passages if the cells are washed twice between passages). If cells are cryopreserved without antibiotics samples may be submitted as is.

3. Other Biological Materials

a) Serum, ascites, or other liquid: 2 vials of at least 0.2 ml/vial shipped on dry ice.

b) Antibodies: It may not be possible to send this sample amount for expensive materials such as antibodies. In this case, a single undiluted aliquot of a reasonable volume can be submitted.

c) Reducing sample volume will reduce analytical sensitivity. Do not dilute sample to reach a specified sample volume.

* For pooling samples: add 200 µl of each sample (media/cells) to two vials.

Please note that it is important that the media is included as virus may be associated with media and not necessarily the cells.

Up to four (4) samples may be pooled (at the pathology laboratory) to reduce costs. If results are negative then the tested samples are Specific Pathogen Free (SPF) and material can be utilised for rodent studies. If results indicate contamination, individual vials representing each of the pooled submissions will be tested separately for the pathogen detected at a cost of ~ $60 per agent per sample.
Biological Materials Form

Biological Material Entering ANU Animal Facilities

This form is required for ALL biological material being used within Animal Facilities. Please complete a new form for each biological. All fields in this form must be completed, indicate n/a if not applicable.

Please email this completed form to animal.shipments@anu.edu.au at least 1 month prior to the commencement of the experiment (allowing more time will allow for samples to be pooled with other shipments to reduce costs).

1. PERSONAL DATA

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<tr>
<th>Date</th>
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<tbody>
<tr>
<td>Researcher name</td>
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2. EXPERIMENT DATA

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3. BIOLOGICAL DATA

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<td>Type (e.g. Cell line, antibody, vaccine, protein)</td>
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<td>Origin (e.g. Rat, mouse, sheep)</td>
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<td>Source/supplier (e.g. ATCC, specific laboratory)</td>
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<td>Were biologicals cultured at any time with rodent serum or fluid? (Y/N)</td>
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<td>If rodent origin has any PCR or other testing been done? (Y/N –if Yes please provide details)</td>
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<td>Other information</td>
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Pathology report number
Biological Health Report Approved by Veterinarian and Facility Management
Copy of results forwarded to animal.ethics@anu.edu.au