Biological Material Screening for Rodent Pathogens

The intent of this standard operating procedure (SOP) is to describe biological material screening and sample submission. This SOP is intended for use by Principal Investigators (PIs), research staff, the Institutional Animal Care and Use Committee (IACUC) and Comparative Medicine (CM). This SOP is approved by the NUS IACUC. Any deviation must be approved by the IACUC in advance.

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1. INTRODUCTION

When biological material is introduced into an animal, it is a potential source of infection by adventitious pathogens. Infection/contamination may also have an impact on cells/biological material, and results of experiments.

Biological materials that are of 1) rodent origin or 2) non-rodent (including human) origin that have been passaged through rodents or exposed to murine products **must** be tested for specific murine pathogens by a CM approved diagnostic laboratory **prior to use in animals**. The negative testing result is a requirement for IACUC protocol approval. Please refer to Appendix A for list of biological material to be tested and Appendix B for the list of murine pathogens to be tested.

For biological material that requires testing, a negative test result is valid for as long as a protocol remains active (maximum 4 years), but only for the same batch of biological material that was tested negative provided it has been appropriately stored (e.g. -80^{o}C freezer, stored in aliquots and thawed as needed).

The use of Matrigel, or any other media of rodent origin or exposed to murine products, in combination with biological material requires submission of the lot-specific Certificate of Analysis (COA) to CM for review and approval prior to use in animals. The COA needs to be submitted along with a Biological Material Testing Request form at the same time.

2. PROCEDURES

a. Submit the completed Biological Material Testing Request (BMT) form to biologicalmaterial@nus.edu.sg.

   i. **Submit BMT Form A (see Appendix C) for protocols created after 4th July, 2018.**

ii. **Submit BMT Form B (see Appendix D) for protocols created prior to 4th July, 2018.**

iii. If Matrigel or other media is used in combination with biological material, submit the lot-specific Certificate of Analysis together with the Biological Material Request form.

b. CM review the form and notifies users of the next step:

i. **BMT Form A:** proceed with sample submission

ii. **BMT Form B:** sample is exempted or requires testing

c. Submission of samples to CM:

i. Collect samples aseptically.

ii. Sample preparation for submission:

1) Cells: Submit two 1 ml cryovials of each cell line sample containing a minimum of 2 x 10^6 cells. Cells can be in pellet form, in freeze media, growth media, or PBS.

2) Liquids: Submit two 1 ml cryovials with at least 0.5 ml.

iii. Label all samples with PI name and sample ID (cell line designation).

iv. Pack samples in leak-proof triple containment (e.g. place tube containing sample (primary) along with absorbent material such as paper towels inside a leak proof zip lock biohazard plastic bag (secondary), which can be placed in a carrier/ice box with lid (tertiary)).

v. Submit samples to CM veterinary technician/staff only.

1) Location: CM pharmacy (by MD2 security on L1)

2) Hours: Monday to Friday 10:30-11:30am and 2:30-3:30pm

vi. CM submit the sample to an approved diagnostic laboratory (e.g. IDEXX BioResearch, Charles River Lab).

d. Allow 3 - 4 weeks from the time of sample submission until results are received. The cost of biological material testing is currently borne by CM.

e. CM notify users and the IACUC office of test results via email.

i. Negative test results: an updated Biological Material Testing Request form indicating negative test results and validity period is sent to users and the IACUC office.

ii. Positive test results: users are required to submit samples from a new batch of biological material for testing. Biological material with a positive test result is not approved for use.

f. For Matrigel and other media, CM review the Certificate of Analysis and notify users and the IACUC office of the validity period. A new Biological Material Testing Request form is required 1) upon expiry of the validity period or 2) if a new/different lot of Matrigel/other media is used.

3. REFERENCES

- IDEXX BioResearch link: http://www.idexxbioresearch.com/biological-testing
- Charles River – Research Animal Diagnostics https://www.criver.com/products-services/research-models- services/animal-health-surveillance/cell-lineresearch-biologics-screening?region=3701
- Cornell https://ras.reserch.cornell.edu/care/documents/ACUPs/ACUP619.pdf
- Biological Materials in Rodents (IACUC), Boston University (revised January 2014) - http://www.bu.edu/researchsupport/compliance/animal-care/working-with-animals/procedures/biological-materials-in-rodents-iacuc/
- NUS OSHE: http://www.nus.edu.sg/osh/index.html

Revision #	Author	Effective Date	SOP #:
.02	Anna Acuna	17 July 2012	605.02
.03	Shannon Heo	20 February 2017	605.03
.04	Jasmin Wu	18 September 2018	605.04

Revision .04: Addition of requirement for using Matrial/media. Revised the procedure to include packaging of sample for submission, time/location for sample submission, and notification of test results. Replaced the BMT request form in Appendix C with the updated form.

4. APPENDICES

Appendix A

Examples of biological materials to be tested include:**	**Examples of biological materials that may not require testing by CM:**
Cell lines originating from rodents	Human cell lines*
Transplantable tumours from rodents	Transplantable tumours/ patient-derived xenografts
Serum, tissues and body fluids from rodents	Serum, tissues and body fluids from humans
Antibody preparations from rodents	Human embryonic stem cells
Hybridomas from rodents	Viruses or viral vectors which have been synthesized without exposure to rodents
Virus originating from rodents or viruses which have been exposed to rodent products directly or indirectly	Bacteria or bacterial products/ components (e.g. LPS, endotoxins)
Basement membrane matrix (e.g. Matrigel®)	Human biologics which have not been passaged through rodents or exposed to rodent products directly/indirectly.
Any non-rodent biologics (e.g. hybridomas, antibody preparations) which may have been exposed to rodents or rodent products directly/indirectly	Murine biological material derived from donor animals and recipient animals, located within CM facility with the same health status.
Biologics originating from hamsters	Commercially obtained biologicals for which the vendor can supply negative screening results, satisfactory to CM.
Human biologics, or cell lines which have been passaged through rodents or exposed to rodent products directly/indirectly.	

Note: This list is not exhaustible.

***** Mycoplasma contamination of cell lines or cell cultures can have undesirable effects on cells (e.g. altered levels of protein, RNA or DNA synthesis, alternation of cellular metabolism, alteration of cell morphology, total culture degeneration and loss etc.), and cause aberrant experimental results. There are various **commercial test kits** available for detection of Mycoplasma spp. in cell cultures and it is recommended that researchers regularly test **human cell lines or cultures** to ensure absence of Mycoplasma contamination.

**All biological materials that require to be tested according to this SOP, must be done through CM (by the approved diagnostic laboratory).
If the biological material has been previously screened for pathogens, the report can be submitted to CM for evaluation of validity of test results and testing laboratory. If CM deemed the test results to be inconsistent with CM requirements, the biological material must be tested again through CM.

Appendix B

Pathogens to be screened

Mouse Pathogens	**Rat Pathogens**
Mycoplasma spp.	Kilham's rat virus
Sendai virus	Toolan's H1 virus
Mouse hepatitis virus	Rat parvovirus
Pneumonia virus of mice	Rat cytomegalovirus
Minute virus of mice	Rat coronavirus
Mouse parvovirus (MPV-1, 2, 3)	Rat minute virus
Theiler's murine encephalomyelitis virus	Sialodacryoadenitis virus
Murine norovirus	Seoul virus
Reovirus-3	*Mycoplasma* spp
Mouse rotavirus	Pneumonia virus of mice
Ectromelia virus	Lymphocytic choriomeningitis virus
Lymphocytic choriomeningitis virus	Sendai virus
Polyoma virus	Mouse adenovirus
Lactate dehydrogenase-elevating virus	Reovirus3
Mouse adenovirus (MAD 1, 2)	Rat Theilovirus
Mouse Cytomegalovirus	
K virus	
Mouse Thymic virus	
Hantaan virus	

Appendix C

Biological Material Testing Request FORM A

Use this form only for protocols created after 4th July, 2018

Date:

Protocol #:

Principal Investigator: **Email/Phone**

Laboratory Contact: **Email/Phone**

In which animal facility and room will the animal(s) receiving this biological material be housed?

Facility **Room**

The biological material is

☐ **A) Rodent-derived:**

To be filled by PI				To be filled by CM	
Name	**Description / Source**	**Media**	**Has this biological been tested previously?**	**Test Results/ Reference Number**	**Validity Period**
Example: Anti-Fas	***Mouse monoclonal antibody to Fas /Commercial***	***PBS***	☐ No		
			☒ Yes (provide reference number: ***IDEXX 2568-2017)***		
1.			☐ No		
			☐ Yes (provide reference number:)		
2.			☐ No		
			☐ Yes (provide reference number:)		
3.			☐ No		
			☐ Yes (provide reference number:)		

OR

☐ **B) Non- rodent (including human) derived, and has been passaged through rodents or exposed to murine products:**

To be filled by PI				To be filled by CM	
Name	**Description / Source**	**Media**	**Has this biological been tested previously?**	**Test Results/ Reference Number**	**Validity Period**
Example: Hep93C	***Human hepatocellular carcinoma line /Commercial***	***PBS***	☒ No		
			☐ Yes (provide reference number:)		
1.			☐ No		
			☐ Yes (provide reference number:)		
2.			☐ No		
			☐ Yes (provide reference number:)		
3.			☐ No		
			☐ Yes (provide reference number:)		

Please refer to CM SOP # 605 Biological Material Screening for Rodent Pathogens for details on how to submit samples.
For further enquiries and/or form submission, please email biologicalmaterial@nus.edu **Reminder: Protocols will not be approved until biological material screening is complete.**

For CM Use Only

CM Vet:
Assessment date(s):
Comments:

Appendix D

Biological Material Testing/ Exemption Request FORM B

Use this form only for protocols created prior to 4th July, 2018

Date:

Protocol #:

Principal Investigator: **Email/Phone**

Laboratory Contact: **Email/Phone**

In which animal facility and room will the donor/recipient animal(s) be housed?

The biological material is

☐ **A) Rodent-derived:**

To be filled in by PI				To be filled in by CM	
Name	**Description / Source**	**Media**	**Tested previously** (Provide date or IDEXX case number)	**Exempted/ Requires Testing**	**Approved**
Example: Anti-Fas	*Mouse monoclonal antibody to Fas /Commercial*	*PBS*			

Is it derived from donor animals located in the same facility and room as the recipients?

Yes ☐

No ☐ **Facility and Room No**.:

OR

☐ **B) Non- rodent (including human) derived:**

To be filled in by PI				To be filled in by CM	
Name	**Description / Source**	**Media**	**Tested previously** (Provide date or IDEXX case number)	**Exempted/ Requires Testing**	**Approved**
Example: Hep93C	*Human hepatocellular carcinoma line /Commercial*	*PBS*			

Has it been passaged through rodents or exposed to murine products?

Yes ☐ **No** ☐

If yes, was it passaged through rodents or exposed to murine products in the same room as the recipients?

Yes ☐

No ☐ **Facility and Room No.** :

Please submit all requests to biologicalmaterial@nus.edu.sg.

Upon notification, **if testing is required,** PI/designee must arrange for sample testing with the CM Veterinary Diagnostic Laboratory (VDL) prior to material administration to animal. Please call VDL at 6516-6410 to arrange for sample submission.

Protocols will not be approved until exemption or testing result is determined.

For CM Use Only

Comments: