

ANIMAL CARE AND USE STANDARD

The Animal Care & Use Standards are designed to provide guidance regarding good practice to institutional animal users and carers, as well as Animal Ethics Committees (AECs), on the care and use of animals for scientific purposes such as research and teaching. The Standards are evidence-based, reflecting current or accepted good practice and allow for the flexibility that is required in research and teaching activities using animals.

LABORATORY ANIMAL HEALTH MONITORING: MOUSE SENTINEL PROGRAMS

This standard has been developed by the University of Melbourne Animal Care & Use Standards Committee, and endorsed by the University of Melbourne Animal Welfare & Ethics Committee.

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1. ASSOCIATED STANDARDS

This standard should be read in conjunction with the following University of Melbourne Animal Care & Use Standards:

- Not applicable

2. SUMMARY

- 2.1 The health status of experimental animals is an important factor for animal welfare and has the capacity to undermine the integrity and validity of research data. It is therefore critical that a robust laboratory animal health monitoring program be established within the facilities responsible for the care of these animals.
- 2.2 A comprehensive health monitoring program serves to outline relevant pathogens, selection of animals and tissues for testing (including sentinel animal regimes), test methods, sampling frequency, and interpretation of results. Its success relies on a co-operative effort from staff to report data, assess results and develop management plans in real time, in consultation with the Animal Facility Managers (AFM) and Animal Welfare Officer (AWO).
- 2.3 Monitoring strategies for detection of disease using sentinel animals is a widely used and useful method for measuring the health status of a larger research colony. Surveillance is targeted to a defined population; this could be at room level, the facility level or defined by genetic status or usage (breeding/stock/experimental).

3. BENEFITS & RISKS

- 3.1 Routine testing and disease surveillance allows AFMs to understand the health status and disease prevalence within a given colony and/or facility.
- 3.2 Comprehensive health monitoring forms the basis for the facility's quality assurance program. This generates information that enables better collaboration between facilities and facilitates the use and sharing of mouse strains among institutions. Failure to undertake disease surveillance may restrict movement of animals between rooms within a single building or across facilities.
- 3.3 If a pathogen is detected, the AFM and/or AWO are able to utilise this information to tailor their response to the specific organism. Investigation, treatment, management and containment plans can be implemented accordingly.
- 3.4 Improved knowledge overall about the microbiological quality of animals used in research and breeding colonies.

- 3.5 Sentinels are a cost-effective way of monitoring mouse colonies for the presence of pathogens. The three major types of sentinel systems available are dirty bedding, direct contact and air-exhaust, with the first two systems being most common. Random sampling and census sampling may also be conducted for smaller areas when required but should first be discussed with the AFM/AWO to ensure animal numbers being tested are appropriate.
- 3.6 Dirty bedding sentinels are most effective at detection of pathogens spread by the faeco-oral route, such as Rotavirus, Mouse Hepatitis Virus, Reovirus, and Helicobacter. It requires relatively few animals compared to direct-contact methods, as 3-4 mice in one cage may be used to sample multiple cages (a maximum of 80 IVC's).
- 3.7 Direct contact sentinels can be used to detect pathogens transmitted by animal to animal contact, aerosol, urine or faeco-oral route. One sentinel mouse is required per cage, and must be housed with the group for a minimum of 4 weeks.
- 3.8 No single method of disease surveillance yields a 100% sensitivity rate for detection of pathogens. For this reason, a screening program should encompass multiple avenues including sentinel systems (dirty bedding, direct contact and random sampling), environmental testing and direct monitoring of subset of colony animals.
- 3.9 Frequency of testing and sample size will vary and may need to be increased to enable detection of pathogens at low prevalence. A combination of serological testing and post mortem sampling will be required and the AFM/AWO should be consulted as to the most appropriate options where specific pathogen identification or follow up is required.

4. PROCEDURE/PROTOCOL

4.1 Mouse selection

- 4.1.1 *Source:* Mice should be sourced from approved, reputable suppliers or facilities able to provide evidence of quality assurance testing, or bred in-house to the same health standards.
- 4.1.2 *Strain:* The Swiss or BALB/c strains are acceptable for use as sentinel mice, however the Swiss strain is preferable due to their more robust immune response. Other strains may be used if deemed suitable following discussion and approval from the AFM and AWO.
- 4.1.3 *Sex:* Female mice should be used as sentinels over male mice as male sentinels housed together have been known to fight causing significant injury. The risk of males fighting becomes greater when exposed to dirty bedding on a weekly basis.
- 4.1.4 *Age:* Mice should ideally be introduced as sentinels at 3-5 weeks of age (and no older than 10 weeks). This is to ensure mice remain immunocompetent and are not subject to age-related diseases at the end of a 3-6 month sentinel period.
- 4.1.5 *Replacement:* Once a sentinel animal has been removed, a replacement sentinel should be added to the group within 1-2 weeks. This will maximise the potential for pathogen exposure and allow the new animal to undergo seroconversion prior to the next round of testing.

4.2 Housing, duration and exposure

- 4.2.1 Sentinels must only ever be co-housed, thus a minimum of 2 will always be present.
- 4.2.2 Sentinel rodents must be housed in the same cage type as the rest of the colony animals, always in a solid based cage and provided with appropriate enrichment (e.g. cardboard rolls, shredded paper, tissue, plastic housing).
- 4.2.3 A cage of sentinels can be housed for a minimum of 8-10 weeks and maximum duration of 6 months.
- 4.2.4 One sentinel cage may service 50 -80 individually ventilated cages (e.g. a standard 100-cage rack, if fully stocked, will require a minimum of 2 sentinel cages and minimum of 4 mice). After this period new animals are required to refresh the sentinel program.
- 4.2.5 Beyond 6 months, sentinel mice may develop signs of disease attributable to chronic immunologic stimulation or age-related changes (e.g. Lymphoma in aged mice). Such disease signs are reflective of the individual and not the health status of the colony and are thus invalid as a population screening tool.

4.3 Dirty bedding transfer

- 4.3.1 Depending on the number of colony cages to be sampled, every cage may be sampled at every cage clean or rotating sampling system may be employed. This should be developed in consultation between the AFM/AWO to maximise the potential for pathogen detection.

- 4.3.2 Sentinel animals should only be handled and their cages cleaned following the cleaning/handling of all other colony animals in the rack or room (This will depend on cage numbers and should be discussed with the AFM). A sample of heavily soiled bedding containing urine and faeces must be collected using a disposable or sterilisable tool such as a plastic cup or spoon from each cage being cleaned.
- 4.3.3 Approximately 5ml (one plastic teaspoon) of dirty bedding from each cage being changed should be placed into a clean, empty cage (without bedding). The total volume of dirty bedding should not exceed 500ml. Contents of the new cage should be mixed evenly using a disposable spoon or tongue depressor for even distribution of waste material.

4.4 Direct contact sentinels

- 4.4.1 Direct contact sentinels are indicated where the supplier of mice does not have a satisfactory quality assurance program or in the event of disease management.

4.5 Documentation and Identification

- 4.5.1 A systematic and clearly documented program should be in place to identify the cages and mice to which the sentinels have been exposed. Easy and rapid traceability is essential in the event a positive result is reported; records must be maintained in a current and accessible manner.
- 4.5.2 Sentinel cages must be clearly labelled and advise that these rodents are not to be used for any research purposes. Cage cards must identify mouse strain, source, date of birth, sex, date of sentinel placement, exposure type (dirty bedding or direct) and individual animal ID numbers.
- 4.5.3 A testing schedule including dates and type of test for each round of sampling should be prepared ahead of time to allow adequate resource allocation to sample collection, storage, testing and animal replacement where necessary.

5. MONITORING & INTERVENTION

- 5.1 During an outbreak or high risk situations the standard testing regime may need to be altered following instructions from the AFM or AWO. This will be established on an individual case basis depending on the facility, set up and pathogen involved.

5.2 Testing

- 5.2.1 Testing and sample collection conducted on-site must only be performed by someone who is competent in the techniques (e.g. Blood collection, skin scrapes, fur plucks, tape preparations).
- 5.2.2 Diagnostic tests should be carried out by an approved laboratory using known and scientifically validated methodologies (e.g. serology, PCR, microbiology).
- 5.2.3 Peri-anal tape preparations for *Syphacia obvelata* can be performed in-house with minimal training and can complement the sentinel program.

5.3 Sentinel Surveillance Program

- 5.3.1 A minimum of twice weekly visual inspections are required for sentinel mice to assess appearance, body condition and behaviour between quarterly testing periods.
- 5.3.2 To allow early signs of disease to be easily identified, an Intervention Criteria Sheet may be prepared in addition to the daily monitoring sheet for the sentinel group. Examples are available on the OREI website.
- 5.3.3 An action plan should be outlined on the sentinel monitoring sheet (or Intervention Criteria Sheet if in use) to enable staff to respond to any changes in sentinel health and give clear instructions for reporting of these findings to the AFM.
- 5.3.4 The AFM and AWO are strongly encouraged to liaise with the laboratory pathologist to maintain a current working knowledge of the pathogens present in a colony, or those pathogens which may have increased in prevalence with each round of testing.
- 5.3.5 Positive test results may restrict the ability of the facility to move animals to collaborating institutions. It is the AFMs responsibility to maintain these records and transmit information where appropriate.

5.4 Suggested testing regime and pathogens

5.4.1 The murine pathogens of high priority are included below, as per the testing standard used by Cerberus Sciences. At a **minimum** facilities should screen for the following agents at the frequencies indicated:

Virus	Test Method	Frequency
Mouse Hepatitis Virus (MHV)	Serology or PCR	Quarterly
Rotavirus (EDIM)	Serology or PCR	Quarterly
Mouse Parvovirus (MPV)	Serology or PCR	Quarterly
Mouse Norovirus (MNV)	Serology or PCR	Quarterly*
Minute Virus of Mice (MVM)	Serology or PCR	Quarterly
Theiler's encephalomyelitis (TMEV)	Serology or PCR	Bi-annual
Pneumonia Virus	Serology or PCR	Bi-annual

Bacteria	Test Method	Frequency
<i>Helicobacter spp</i> *	PCR	Quarterly *
<i>Pasteurella pneumotropica</i> *	Culture and PCR	Quarterly *
<i>Bordetella bronchiseptica</i>	Culture	Bi-annual
<i>Klebsiella pneumoniae</i>	Culture	Bi-annual
<i>Streptococcus pneumoniae</i>	Culture	Bi-annual
<i>Streptobacillus moniliformis</i>	Culture	Bi-annual
<i>Pseudomonas aeruginosa</i>	Culture	Bi-annual
<i>Corynebacterium kutscheri</i>	Culture	Bi-annual
<i>Citrobacter rodentium</i>	Culture and PCR	Bi-annual
<i>Salmonella spp</i>	Culture	Bi-annual

* If present on initial screen and no effort is made at eradication then testing frequency should be reduced to bi-annual or annual.

Parasites	Test method	Frequency
GI Worms	Wet mount/Tape/Faecal float	Quarterly
Ectoparasites	Direct/Tape	Bi-annual
<i>Entamoeba muris</i>	Wet Mount	Bi-annual
<i>Giardia spp</i>	Wet Mount	Bi-annual
<i>Spironucleus spp</i>	Wet Mount	Bi-annual
<i>Eimeria spp</i> (intestinal)	Wet Mount	Bi-annual
<i>Pneumocystis carinii</i>	PCR (Lung at autopsy)	Annual (immunocompromised only)

5.5 The decision to test for these pathogens or any additional ones that are not listed should be made in consultation with the AFM and AWO. This may be influenced by previous test results, budget allocations, what is most relevant to the colony under surveillance and individual facility quarantine requirements.

5.6 Facilities that have recorded a positive result or are known to have a problem with a particular pathogen should increase the test frequency and implement random sampling into the testing regime to enable greater sensitivity when looking for low levels of an organism.

6. ADDITIONAL INFORMATION

6.1 Specific information relating to testing protocols and samples required should be discussed either with the laboratory responsible for the testing or the AWO.

6.2 In the event of a positive test result a management plan should be formulated following joint consultation with the AFM and AWO.

7. ENFORCEABLE REQUIREMENTS

7.1 Only trained competent technicians can be responsible for the sentinel's husbandry.

7.2 Sentinels must be housed in social groups, provided with enrichment and monitored closely for any signs of illness. Prompt reporting to the AFM/AWO and appropriate intervention is essential.

7.3 Announcing and reporting to collaborative facilities when new positive results are reported.

7.4 Use of a University of Melbourne accredited laboratory service to conduct all required testing (e.g. Cerberus Laboratories).

8. EXEMPTIONS

Where adherence to this Standard conflicts with proposed work, the University's AECs may grant exemptions to all or part of the Standard. To seek exemption, applications should clearly outline how the proposed work deviates from the Standard, and justify the need for this. Before seeking exemption, it is recommended that you consult with the University's AWO.

9. UNEXPECTED ADVERSE EVENTS

An unexpected adverse event is any event, which impacts negatively on the wellbeing of animals, and which was not anticipated, or has occurred at a frequency or severity in excess of what was anticipated in line with the AEC approval. This can be a single or cumulative event, and will normally involve unexpected mortality, morbidity or injury. Anyone identifying an unexpected adverse event must act to remove and/or minimise any immediate risk to animals. Immediately thereafter, the University's AWO and relevant AFM must be notified of the event. The AWO will advise researchers of the appropriate response.

10. GLOSSARY

Scientific Term	Lay Description
Sentinel	Mice that are exposed to similar housing and environmental conditions to all other animals in the research group. Results of disease screening from these 1-2 animals are extrapolated to indicate disease in the wider population.
Pathogen	Any disease-producing agent, especially a virus, bacterium, or other microorganism.
Serology	The science dealing with the immunological properties and actions of serum.
Antigen	The portion of a pathogen that is recognisable to the immune system. Used as a focus to eliminate disease or can be detected in tests to indicate a disease is present.
Antibody	The unit of the immune system able to neutralise a particular pathogen. May be generated by natural exposure to an antigen or vaccination. Presence of antibody indicates disease was or is present if naturally occurring.
Virus	An ultramicroscopic (20 to 300 nm in diameter), metabolically inert, infectious agent that replicates only within the cells of living hosts, mainly bacteria, plants, and animals: composed of an RNA or DNA core, a protein coat, and, in more complex types, a surrounding envelope.
Bacteriology	The branch of science concerned with the study of bacteria.
Bacteria	A very large group of microorganisms comprising one of the three domains of living organisms. They are prokaryotic, unicellular, and either free-living in soil or water or parasites of plants or animals.
Parasitology	The branch of biology that is concerned with the study of parasites.
Ectoparasite	A parasite, such as the flea, that lives on the outer surface of its host.
Endoparasite	A parasite, such as the tapeworm, that lives within the body of its host.
Pathological	Relating to, involving, or caused by disease.
Microbiological	Microorganisms, including algae, bacteria, fungi, viruses, and protozoa.
Seroconversion	The process by which a hosts immune system generates antibodies (immunity) in response to exposure to a pathogen. Seroconversion takes time and is needed before many tests are able to detect an animal has been exposed to an organism or is currently infected.

11. REFERENCES & RESOURCES

The following source material contributed to the development of this Standard:

- Mähler, M., Berard, M., Feinstein, R., Gallagher, A., Illgen-Wilcke, B., Pritchett-Corning, K., & Raspa, M. (2014). FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units. *Laboratory Animals*, 48(3), 178-192.
- Compton, S. R., Homberger, F. R., & MacArthur, C. J. (2004). Microbiological monitoring in individually ventilated cage systems. *Lab animal*, 33(10), 36-41.
- Pritchett-Corning, K. R., Prins, J. B., Feinstein, R., Goodwin, J., Nicklas, W., & Riley, L. (2014) A Harmonized Health Reporting Format for International Transfer of Rodents. *JAALAS*. 53: 633-640
- Voipio, H. M., Baneux, P., de Segura, I. G., Hau, J., & Wolfensohn, S. (2008). Guidelines for the veterinary care of laboratory animals: report of the FELASA/ECLAM/ESLAV Joint Working Group on Veterinary Care. *Laboratory animals*, 42(1), 1-11.

The following resources may provide additional or supplementary information:

- ESLAV, European Society of Laboratory Animal Veterinarians. See <http://www.eslav.org/>