Rodent Health Monitoring

Standard Operating Procedures

Purpose: Rodent health monitoring programs are designed to detect subclinical infections of rodents that have the potential to cause detrimental effects to research. The purpose of this SOP is to provide a minimum guideline for Purdue University’s health monitoring program. Testing is conducted at a frequency based on the probability of contamination with specific agents and at sufficient intervals to reduce the spread of infection within and between facilities if contamination occurs.

Selection of sentinel animals:

Sentinel animals must be selected from strains that are immunocompetent, susceptible to viral infections and develop strong antibody responses when exposed to potential rodent pathogens. Mouse strains that meet the criteria are typically outbred strains such as CD-1 mice. Rat strains that meet the criteria are outbred strains such as Sprague Dawley. Purdue University typically uses CD-1 mice and Sprague Dawley rats for sentinel animals in rat and mouse colonies. Guinea pigs will typically be tested using samples from colony animals that are purchased for research/teaching projects.

Areas selected for sentinel monitoring will be those housing long-term (greater than 6 months) colonies of mice, rats and guinea pigs. This will include areas where rodents are replaced frequently but the area is in constant use.

Selection of agents to be screened:

Selection of agents to be screened will be limited to those more commonly encountered in laboratory rodents, are highly contagious, or potentially affect research results. There are two levels of screening: annual testing and trimester testing. The decision whether to test annually or every trimester is based on the frequency of positive test results and the requested test frequency that will allow transfer of rodents to other institutions. Transfer of rodents originating from Purdue University typically pertains to unique genetically modified mice only.

Procedure for Sentinel Animal Testing:

A. Placement of Sentinels within the Designated Housing Areas

1. Each designated rack equipped with caging is provided with one sentinel cage per side of rack. In cases where under 80 cages are present one sentinel cage may be used for the whole rack or whole room.

   a. Each cage will contain 2 CD-1 female mice or 2 female SD rats from Envigo. Males may be substituted only if required by the PI in the room for study purposes.

   b. Sentinel cages will be placed on the bottom shelf right corner of either the rack of static microisolators or the ventilated rack being monitored.

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2. Sentinel cages will be changed completely at normal cage changing frequency of the experimental group that is being monitored.

3. Sentinel cage cards will be clearly identified with the room number, rack number, PIs of colony animals, and will be dated and labelled when bleeds occur.

**B. Procedures for Providing Sentinels Soiled Bedding**

1. The animal caretaker changing the cages in the designated area is responsible for providing the sentinel mice with a mixture of soiled bedding (from cages in the same rack as the sentinel mice) with or without fresh bedding.

2. The sentinel cages are changed last. The caretaker collects soiled bedding by placing an extra cage in the hood in which half of the bedding has been removed. Approximately 1 teaspoon of dry soiled bedding (including feces) should be taken from every cage on one side of the rack and placed into the empty cage in the hood. The spoon should be disinfected in between sentinel cages. The fresh bedding in the cage may be removed in order to prevent overfilling the cage. If the cage is still too full, the soiled bedding may be mixed, then a portion removed prior to placing the sentinel mice in the cage.

3. Once all the cages have been changed, the sentinel animals are then placed into the cage containing the soiled bedding.

**C. Procedures and Schedule for Testing Sentinel Rodents**

1. **Procedure for Mouse Sample Collection Trimesters 2 & 3**
   a. One mouse per sentinel cage is bled by submandibular or submental collection techniques.
   b. The blood is collected on a blood spot card or serosorb device.
   c. The bled mouse is then ear-punched and returned to the cage
   d. The cage card is labelled with the date and ear punch ID
   e. Fur swabs of each sentinel mouse and fresh fecal sample from each mouse in each sentinel cage in the room are pooled. 10-14 total fecal pellets and fur swabs may be pooled for each room.
   f. Fecal pellets must be stored in the freezer until submission, and shipped on ice.

2. **Procedure for Mouse Sample Collection Trimester 1**
   a. The mouse NOT ear-punched per sentinel cage is taken alive to the LAP lab for sampling.
   b. The mouse is euthanized by either CO2 exposure or IP barbiturate overdose.
   c. Each mouse per sentinel cage has blood collected by cardiac puncture or submental collection methods onto a blood spot card or serosorb device.

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d. Fur swabs of each sentinel mouse and fresh fecal sample from each mouse in each sentinel cage in the room are pooled. 10-14 total fecal pellets and fur swabs may be pooled for each room.

f. Fecal pellets must be stored in the freezer until submission, and shipped on ice.

g. A necropsy is performed and gross examination of all major organs is completed. Any abnormal lesions, growths or discolorations will be noted accordingly.

h. Gloves should be changed between handling sentinels from different rooms.

i. After results have been obtained, the remaining mouse in each sentinel cage will be euthanized by animal care staff with CO2 exposure and new animals will be ordered.

3. Procedure for Rat Sample Collection Trimester 1 only

a. One rat per sentinel cage is taken alive to the LAP lab for sampling.

b. The rat is anesthetized with isoflurane then euthanized by either CO2 exposure or IP barbiturate overdose.

c. Each rat per sentinel cage has blood collected by cardiac puncture onto a blood spot card.

d. Fur swabs of each sentinel rat and fresh fecal sample from each rat in each sentinel cage in the room are pooled. 2-6 total fecal pellets and fur swabs may be pooled for each room.

f. Fecal pellets must be stored in the freezer until submission, and shipped on ice.

g. A necropsy is performed and gross examination of all major organs is completed. Any abnormal lesions, growths or discolorations will be noted accordingly.

h. After results have been obtained, the remaining rat in each sentinel cage will be euthanized by animal care staff with CO2 exposure and new animals will be ordered.

*In the event that the Transgenic core needs rats live bled on Trimesters 2 & 3, blood collection via the saphenous or tail vein routes may be used to collect blood for blood spot card or serosorb device submission.

4. Procedure for Rat Parasitology Trimesters 2 & 3 only

a. Fur swabs of each sentinel rat and fresh fecal samples from each rat in each sentinel cage in the room are pooled. 2-6 total fecal pellets and fur swabs may be pooled for each room.

b. Fecal pellets must be stored in the freezer until submission, and shipped on ice.

5. Procedure for Direct Colony Animal Parasitology Sample Collection

a. On a trimester basis, fur swabs will be taken from random cages for both mice and rats in each room using the schedule below. One fur swab will be used to swab all animals in one cage, and one cage per

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investigator on each side of every rack in the room will be tested. A new swab will be used per cage, then swabs for each room will be pooled into a 5ml tube for submission.

b. Facility schedule is T1- Biology, T2- Nutrition, Animal Science, Hanson, and Vet Med, T3- Bindley, Jischkey, Psychology, Pierce, Pharmacy

6. Exhaust Air Environmental Testing Sample Collection

a. EAD filters may be used in place of sentinel animals on Allentown racks.

b. The racks must be sanitized every 12 months.

c. Filters will be placed at the top of the vertical exhaust manifold and left in place for at least 3 months prior to testing.

d. Filters will then be replaced, and the samples will be placed in a 50ml conical tube and submitted.

e. On a trimester basis, fecal samples will be collected from random cages for mice on each EAD rack using the schedule below. One sample will be collected from ~10 cages per room, making sure to collect a sample from each side of every rack and each PI. This sample will be pooled, frozen, and submitted on ice for virology testing.

f. Facility schedule is T1- Biology, T2- Nutrition, Animal Science, Hanson, and Vet Med, T3- Bindley, Jischkey, Psychology, Pierce, Pharmacy

7. Environmental Sampling of Static or Cage Level Filtered Cages

a. Environmental PCR samples may be used in place of live sentinel animals for static cages or cages with filtration at cage level (Bindley cubicle racks).

b. Two swabs are placed in a clean cage without bedding

c. At every cage change, dirty bedding is placed in the cage as for a normal sentinel cage. The bedding is allowed to accumulate for at least 12 weeks.

d. At every cage change, when dirty bedding collection is complete, a lid should be placed on the cage and the cage is to be shaken 3 times, then placed back on the rack/shelf.

e. The lid should be left off if cages in the room are open-air, or left on if biohazards or the other cages have lids.

f. After at least 12 weeks, the swabs are to be collected, swabbing the inside of the cage walls prior to placing in a 1ml collection tube together for PCR submission.

g. On a trimester basis, fecal samples will be collected from random cages for mice on each rack using the schedule above. One sample will be collected from ~10 cages per room, making sure to collect a
sample from each side of every rack and each PI. This sample will be pooled, frozen, and submitted on ice for virology testing.

In the event that a sentinel animal dies or is euthanized due to health issues, if possible it should be sent to ADDL for a full necropsy. In addition, a new sentinel may be placed in the cage and identified with an **ear punch in both ears**, and the cage card labelled to reflect the date the animal was added.

**D. Mouse Sample Submission to VRL and Agents Tested:**

1. **Trimester 1**
   a. Purdue Mouse Comprehensive Serology Panel (blood) - MHV, MVM, NS1, MPV 1-5, MNV, TMEV, EDIM, Sendai, *M. pulmonis*, PVM, REO3, LCMV, Ectro, MAV 1, MAV 2, Polyoma, Helico
   b. Purdue Basic Parasite PCR Panel – Frozen fecal, fur swab
   c. Direct Fur swab sample pooled by room for T1 facilities- single agent PCR assay for fur mites

2. **Trimesters 2, 3**
   a. Purdue Mouse Quarterly Serology Panel- MHV, MVM, MPV 1-5, MNV, TMEV, EDIM
   b. Purdue Basic Parasite PCR Panel – Frozen fecal, fur swab
   c. Direct Fur swab sample pooled by room for T2 or 3 facilities- single agent PCR assay for fur mites

**E. Rat Sample Submission to VRL and Agents Tested:**

1. **Trimester 1**
   b. Purdue Basic Parasite PCR Panel – Frozen fecal, fur swab
   c. Direct Fur swab sample pooled by room for T1 facilities- single agent PCR assay for fur mites

2. **Trimester 2 & 3**
   a. Purdue Basic Parasite PCR Panel – Frozen fecal, fur swab
   b. Direct Fur swab sample pooled by room for T2 or 3 facilities- single agent PCR assay for fur mites

**F. Environmental Testing - Mouse**

1. **Trimester 1**

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a. Purdue Mouse Comprehensive Filter Panel - *M. pulmonis, Helicobacter* spp, MHV, MVM, MPV 1-5, MNV, TMEV, EDIM, MAV 1, MAV 2, REO3, PVM, Sendai, Ectro, Hantaan, LCMV, Pinworms, Fur mites, Poly

b. Purdue Mouse Fecal PCR Panel- (frozen fecal sample pooled by mouse room) MHV, MPV, MVM, MNV, EDIM for T1 facilities

2. Trimesters 2 & 3

a. Purdue Mouse Quarterly Filter Panel- MHV, MVM, MPV, MNV, TMEV, EDIM, Pinworms, Fur mites

b. Purdue Mouse Fecal PCR Panel- (frozen fecal sample pooled by mouse room) MHV, MPV, MVM, MNV, EDIM for T2, or 3 facilities

**G. Environmental Testing - Rat**

1. Trimester 1


2. Trimesters 2 & 3

a. Purdue Rat Basic Filter Panel- Helicobacter, RCV/SDA, RPV, KRV, H-1, RMV, RTV, Pinworms, Fur mites

**H. Guinea Pigs**

a. Direct colony testing may be performed biannually for *Clostridium piliforme*, CavPI3, LCMV, PVM, Sendai, *Encephalitozoon cuniculi*, pinworms, and fur mites as needed

All samples will be sent to an appropriate laboratory for testing. Appropriate laboratories include VRL, IDEXX Bioresearch, Charles River Laboratories and the Animal Disease Diagnostic Laboratory.

Necropsies will be performed by Purdue University Laboratory Animal Veterinary Staff.

Results from all testing will be distributed by email to all principal investigators housing rodent species tested, facility managers, facility directors, IACUC chairperson, and Laboratory Animal Program Veterinary staff.

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