University Animal Care Committee Standard Operating Procedure		
Document No: 14.4	**Subject:** Rodent Health Monitoring	
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Location: Queen's University

Responsibility: Principal Investigators (PI), Research Staff, Veterinary Staff

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe the Health Monitoring Program of all rodent colonies at Queen's University. The health status of rodents needs to be monitored on a continual basis to detect any infectious disease outbreak within the colonies of mice, rats and other rodents. Rapid detection of diseases enables the Animal Care team to prevent disease transmission within the facilities and from external sources.

1. **Introduction and Definitions:** Pathogenic agents including viruses, bacteria and parasites can contaminate the colonies and affect the rodent's health status. These clinical and/or subclinical pathogens can confound results of experimental studies being carried out on these animals.

2. **Materials:**
   - Ketamine (100mg/ml)
   - Xylazine (10mg/ml)
   - Sterile water
   - Isoflurane
   - Sterile saline
   - 1cc syringe + 25g needle
   - 1cc syringe
   - 3cc syringe
   - 26g, 23g and 21g needles
   - Microtainers for serum separation
   - 3ml red top vacutainers
   - 1.5-2.0ml Eppendorf tubes
   - 2ml sterile Eppendorf tubes
   - 5ml sterile Eppendorf tubes
   - Scalpel handle and # 10 blades
   - Gloves
   - Sterile scissors and forceps
   - Culture swabs (blue for tracheal swabs and red for cecal swabs)
   - Fecal flotation supplies
   - Microscope slides and coverslips
   - Clear scotch tape for pinworm testing

3. Procedures:

Surveillance Frequency

- Rodent sentinels will be tested quarterly.
- Exceptions include short term rodent colonies of less than 6 weeks. Those rodents would be housed separately from long-term colonies.
- Short-term colonies will be evaluated by the University or Clinical Veterinarian to determine if they are exempt from surveillance depending on their health status, use and origin.

Origin of Sentinel Animals

- Sentinel rodents will originate from approved commercial rodent vendors and be free excluded pathogens.
- Approved vendors include Jackson Laboratories, Charles River Laboratories, Taconic and Harlan.

Gender of Sentinel Animals

- Female mice and rats are preferred due to the reduced incidence of aggression.
- Male rats will be used when it is believed females housed in the same room as males will impact a study protocol.

Strain and Age of Sentinel Animals

- Outbred strains of mice and rats are used due to their robust immune response.
- Inbred mouse and rat strains may be used if they are determined acceptable by the University Veterinarian.
- Rats are ordered at 3-4 weeks of age.
- Outbred mice are ordered at 4-5 weeks of age, dependent on health history and where they are going.

Number of Sentinels and Identification

Rats

- There will be one cage of two pair-housed rats in every holding room. This results in one sentinel cage per 20-50 rat cages.
- First quarter, one rat will be used for a terminal blood collection by cardiac puncture.
- Next quarter, the second rat will undergo a terminal blood collection by cardiac puncture.
- The first and third quarter will have two rats housed in each holding room. The second and fourth quarter will have only one rat sentinel housed in each holding room.

Mice

- There will be a minimum of one cage of two co-housed mice for each ventilated rack or conventional mouse room. This results in one sentinel cage per 50-120 mouse cages.
- Two sentinels are used per cage.
- All quarterly mouse testing is terminal.
- A replacement sentinel is added to each existing sentinel box. Mice will be ear punched to identify newest addition to sentinel box.
- Replacement sentinels must be briefly monitored to ensure no aggression takes place from original sentinels. Hostility usually occurs immediately after the addition of the newest sentinel. If this occurs, removal of the newest sentinel is immediate and this mouse is paired with another mouse from the same intake.
- Replacement sentinels should be placed within 1 to 2 weeks to maximize the exposure period for the next quarter.

Housing and Handling

- Sentinel rodents will be housed in the same type of caging as the housing in the room.
- Sentinel cages should be placed on the lowest shelf of the rack under the experimental rodents.
- Once a group of sentinels has been placed in a room and or on a particular rack, they must remain with the same group of animals.
- Sentinels are cared for last within their testing group.

Exposure

- Sentinel rodents are housed on a composite sample of 100% dirty bedding from colony cages for a minimum period of 5 weeks from the date of first exposure.
- Every time cages are changed, one teaspoon of dirty bedding is removed from each experimental cage and placed in a clean cage with no bedding to be used for the sentinels.
- This will ensure that the sentinels are exposed to any potential pathogens in the room.
- The oldest sentinel will always be taken for testing unless a health problem necessitates the removal of the younger sentinel from the box.
- Animals are taken to another location for testing procedures to be completed.
- Rodents being tested using ‘PRIA’ do not need the minimum 5 week exposure. Fecal collection for testing may be done as early as 3 days post arrival into clean quarantine. Fecal collection is done in the room.

Diagnostics

Serology Sample Collection

- Mice are anesthetized as per SOP 7.6 “Anesthesia in Mice”.
- A cardiac puncture is performed on the mice as per SOP 7.10 “Blood Collection in Mice”.
- Rats are anesthetized as per SOP 10.6 “Anesthesia in Rats”.
- A cardiac puncture is performed on rats as per SOP 10.10 “Blood Collection in Rats”.

- Blood is collected into the appropriate sized collection tube for serum collection.
    - Microtainer serum separator tubes are the preferred for mouse blood collection.
- Serum is collected and frozen (if not being shipped out for testing within 24 hours).

Microbiology

- Upper respiratory culture. Once blood is collected and the animal is euthanized it is placed in dorsal recumbency. Using aseptic technique, a midline incision is made over the neck and the trachea is exposed. Using a 1cc syringe and 26G needle, the trachea is flushed with 0.1ml sterile saline. The contents of the flush are deposited onto a blue top BBL culture swab.
- Cecum culture. Using aseptic technique, make a midline abdominal incision and exposes the cecum. Nick the cecum with the scalpel blade and make an opening to insert a red top BBL culture swab.

PCR Fecal Sample Collection

- For Helicobacter testing, fresh feces are collected into a sterile container using aseptic technique. A pooled sample may contain up to 10 fecal pellets. The pellets should be free of any contamination such as bedding or hair from the animal's cage. Mouse pellets are collected into a 2ml snap top vial and rat pellets into a 5ml snap top vial, ensuring the vial is no more than 75% full. The samples must be refrigerated or frozen. It is preferable to ship the fecal samples within 24 hours of collection on ice packs or dry ice.

PRIA Testing Sample Collection

- Fecal samples. Are collected as for Helicobacter testing.
- Body swabs. Swabs with a pink sticky tip are used to swab the entire body of the rodent, going against the direction of their hair growth. The swab head is cut from the shaft and placed in a vial no more than 75% full. Up to 10 swabs can be pooled and submitted in one vial.
- Oral swab. Small dry swabs with no transport media are used to swab the oral cavity. One swab may be used for up to five animals or up to 10 individual swabs may be pooled for testing. The swab tip is removed from the shaft and placed in a vial no more than 75% full.

Parasitology Procedures

- Anal tape tests will be performed on all sentinels. A piece of clear tape is applied to the anal area of the animal and then placed on a microscope slide. The slide is then examined under a microscope at a minimum of 40x magnification.
- Fur pluck examination: Using hemostats pluck fur from the dorsum of the animal, the head, neck, back or flanks. The fur pluck is placed on a microscope slide with a drop of mineral oil and covered with a glass coverslip. Examine the slide using 10x and 40x magnification.
- Skin scraping: Deeply scrape the skin with a # 10 scalpel blade in the opposite direction of the hair growth to erode the epidermis. After placing a drop of oil on a microscope slide, apply the sample to the oil drop and top with a coverslip. Examine the slide using 10x and 40x magnification.

- Fecal flotation: Once blood is collected and the animal is euthanized make a midline abdominal incision and expose the cecum. Empty contents of cecum into flotation vial, fill vial with Fecasol solution (Sodium Nitrate Solution), mix contents and add a coverslip. Wait 15 minutes, remove coverslip and place on a microscope slide. Examine slide at 10x and 40x magnification.
- Fresh feces may be collected from live animals for testing.

Housing of Quarantined Animals

- Quarantined rodents should be housed in ventilated cages or closed micro-isolator boxes, away from colony animals.
- Rodents housed in 'Clean Quarantine' will be tested by means of 'PRIA' or using the conventional method of exposure to dirty bedding.
- Rodents housed in 'Dirty Quarantine' will be tested using the conventional method of exposure to dirty bedding. Mice in 'Dirty Quarantine' will be re-derived but it is necessary to know what pathogens exist in case of positive results elsewhere in the facility.
- Personnel working in 'Dirty Quarantine' will not be allowed entry into clean rodent rooms for 24 hours.

Positive Results

- A positive result will be reported to the University Veterinarian, Clinical Veterinarian and Facility Manager immediately.
- The affected colony will be quarantined.
- The second sentinel from the cage will be tested.
- The University Veterinarian will decide whether to send the second sample to the same testing facility or a different diagnostic testing laboratory.
- Once the outbreak is confirmed, the University Veterinarian will make the decision to either euthanize the colony or move the rodents to 'Dirty Quarantine'.
- Steps will be taken to identify the source of the pathogen and eliminate/control the infectious agent.

Entry and Quarantine of Rodents from Non-Commercial Sources

- Non-commercial sources include other universities, research facilities and medical institutes national and international.
- Health reports of rodents from these sources must be sent prior to shipment of any animals. The University or Clinical Veterinarian will review these reports and decide whether to accept these rodents into the facility.
- If approved, a decision will be made on the animal use and length of time these rodents will remain in the facility.
- The housing location of these rodents will be decided.
- Serology testing for the animals will be scheduled, if re-derivation is required.

Testing Schedules

Mouse

Schedule	3 months	6 months	9 months	12 months
	March	June	September	December
Barriers	Microbiology: Upper respiratory and cecum culture. Serology: Tracking PCR: Helicobacter All tests above done at Charles River. In house testing: Fecal flotation, pinworm anal tape test and skin scraping for mites.	Microbiology: Upper respiratory and cecum culture. Serology: Assessment PCR: Helicobacter All tests above done at Charles River. In house testing: Fecal flotation, pinworm anal tape test and skin scraping for mites.	Microbiology: Upper respiratory and cecum culture. Serology: Tracking PCR: Helicobacter All tests above done at Charles River. In house testing: Fecal flotation, pinworm anal tape test and skin scraping for mites.	Microbiology: Upper respiratory and cecum culture. Serology: Assessment PCR: Helicobacter All tests above done at Charles River. In house testing: Fecal flotation, pinworm anal tape test and skin scraping for mites.
BioBubble	Serology: Tracking PCR: Helicobacter All tests above done at Charles River. In house testing: Fecal flotation, pinworm anal tape test and skin scraping for mites	Serology: Assessment All tests above done at Charles River. In house testing: Fecal flotation, pinworm anal tape test and skin scraping for mites	Serology: Tracking PCR: Helicobacter All tests above done at Charles River. In house testing: Fecal flotation, pinworm anal tape test and skin scraping for mites	Serology: Assessment All tests above done at Charles River. In house testing: Fecal flotation, pinworm anal tape test and skin scraping for mites
Conventional rooms	Serology: Tracking PCR: Helicobacter All tests above done at Charles River. In house testing: Fecal flotation, pinworm anal tape test and skin scraping for mites	Serology: Assessment All tests above done at Charles River. In house testing: Fecal flotation, pinworm anal tape test and skin scraping for mites	Serology: Tracking PCR: Helicobacter All tests above done at Charles River. In house testing: Fecal flotation, pinworm anal tape test and skin scraping for mites	Serology: Assessment All tests above done at Charles River. In house testing: Fecal flotation, pinworm anal tape test and skin scraping for mites

* All quarterly mouse testing is terminal. Replacement sentinels for each box.

Rat

Schedule	3 months	6 months	9 months	12 months
	April	July	October	January
Botterell Hall Humphrey Hall BioSciences Gidru	Serology: Tracking Testing done at Charles River. In house testing: Fecal flotation, pinworm anal tape test and skin scraping for mites. Terminal Replacement sentinels.	Serology: Assessment PCR: Helicobacter Testing done at Charles River. In house testing: Fecal flotation, pinworm anal tape test and skin scraping for mites. Non terminal	Serology: Tracking Testing done at Charles River. In house testing: Fecal flotation, pinworm anal tape test and skin scraping for mites. Terminal Replacement sentinels.	Serology: Assessment PCR: Helicobacter Testing done at Charles River. In house testing: Fecal flotation, pinworm anal tape test and skin scraping for mites. Non terminal

Mouse Serology Panels

Tracking	MPV, MVM, Parvovirus, MHV, MNV, TMEV (GDVII), EDIM, SEND, PVM, REO, MPUL
Assessment	MPV, MVM, Parvovirus, MHV, MNV, TMEV (GDVII), EDIM, SEND, PVM, REO, MPUL, LCMV, MAV, ECTRO, K, POLY

Mouse Microbiology

Upper Respiratory Culture	B. bronchiseptica, C. kutscheri, K. oxytoca, K. pneumonia, Pasteurella sp., P. multocida, P. pneumotropica, Pseudomonas sp., Ps. Aeruginosa, Staph. Aureus, Strep. Pneumonia, Beta Strep. Sp.-Group B and G, Beta Strep. Sp., Other
Cecum Culture	Ps. Aeruginosa, Pseudomonas sp., Salmonella sp., Other

PCR: Mouse and Rat

Helicobacter Screen	Helicobacter genus, H. bilis, H. ganmani, H. hepaticus, H. mastomyrinus, H. rodentium, H. typhlonius

Mouse Surveillance + PRIA

MHV	Beta Strep Grp G	Helicobacter genus
MPV/MVM	B. bronchiseptica	K. oxytoca
MRV (EDIM)	Campylobacter Genus	K. pmeumoniae
MNV	CAR Bacillus	M. pulmonis
TMEV/GDVII	C. rodentium	P. pneumotropica-Heyl
MAV 1 & 2	C. piliforme	P. pneumotropica-Jawetz
Beta Strep Grp B	C. bovis	Pneumocystis
Beta Strep Grp C	C. kutscheri	Ps. aeruginosa
Salmonella Genus	Pinworm PCR	Spironucleus muris
S. aureus	Mite PCR	Cryptosporidium
S.xylosus	Giardia PCR	
S. moniliformis	S. pneumoniae	

Rat Serology Panels

Tracking	RPV, H-1, KRV, RMV, Generic Parvovirus NS-1, SDAV, RTV, PCAR (“RRV”), SEND, PVM, REO, MPUL
Assessment	RPV, H-1, KRV, RMV, Generic Parvovirus NS-1, SDAV, RTV, PCAR (“RRV”), SEND, PVM, REO, MPUL, LCMV, MAV