RUTGERS UNIVERSITY Office for Research

CMR STANDARD OPERATING PROCEDURE

TITLE: Rodent Health Surveillance Program – Sentinel Animal		
SOP Category: Veterinary	**CMR SOP #: 7.12**	**Page: 1 of 5**
Effective Date: 09/03/2024	**Approval:**	
Revisions: 11/19/2021, 05/18/2022, 08/2024		

SCOPE:

This document describes the procedures to be followed when performing Rodent Health surveillance where live Sentinel animals are to be utilized. This SOP applies to all Animal Care Staff (ACS), ACS Supervisors (ACSS), Veterinary Staff (VS) at the Rutgers University facilities.

OBJECTIVE:

The program is designed to monitor the health status of rodent colonies and detect clinical and subclinical infections that could potentially compromise animal welfare, jeopardize the validity and reproducibility of research data, or have zoonotic concerns.

PROCEDURES:

Health Monitoring Program Overview

The health monitoring program may require use of animal-based testing methods. Animal based monitoring utilizes serial soiled bedding exposure of sentinel mice with periodic blood/pelt/feces sampling. Serological antibody testing is use for blood sample testing. Polymerase Chain Reaction (PCR) is used for testing all non-blood samples.

Animal Based Monitoring:
Soiled (dirty) bedding exposed sentinel mice are monitored three times a year (every 4 months). All testing is performed externally through a diagnostic lab (e.g. Charles Rivers Laboratories). Pelt swabs and fecal PCR are performed for endo/ecto parasites, and these samples are generally batched by zone (multiple cages or rooms). Serology testing alternates between Prevalent and Assessment Plus MFIA panels.

Two female, CD-1 mice or CD rats are placed in one cage. Sentinel ratios are maintained at one cage per 70-160 rodent colony cages. Sentinel cages may represent one or more researcher colonies depending on the facility. Sentinels are replaced every 8 months. Sentinels must be maintained in the facility for at least 8 weeks prior to testing. All sentinel animals are monitored based on the following schedule:

Testing Schedule

Testing Month	January (Survival)	May (Euthanize†)	September (Survival)	January (Euthanize)	May (Survival)	September (Euthanize)
Serology Panel	MFIA Prevalent* Profile	MFIA Prevalent Profile	MFIA Assessment* Plus Profile	MFIA Prevalent Profile	MFIA Prevalent Profile	MFIA Assessment Plus Profile
PCR Panel	Pinworm/Fur Mite PCR (Helicobacter PCR, if required)	Pinworm/Fur Mite PCR (Helicobacter PCR, if required)	Pinworm/Fur Mite PCR (Helicobacter PCR, if required)	Pinworm/Fur Mite PCR (Helicobacter PCR, if required)	Pinworm/Fur Mite PCR (Helicobacter PCR, if required)	Pinworm/Fur Mite PCR (Helicobacter PCR, if required)
Required Sample Type	HemaTip, Pooled fecal pellets, pooled fur swabs	HemaTip, Pooled fecal pellets, pooled fur swabs	HemaTip, Pooled fecal pellets, pooled fur swabs	HemaTip, Pooled fecal pellets, pooled fur swabs	HemaTip, Pooled fecal pellets, pooled fur swabs	HemaTip, Pooled fecal pellets, pooled fur swabs

*Charles River Testing Profiles
†All sentinels are euthanized, processed, and replaced

A necropsy should be performed on all euthanized or dead sentinels. When sentinels are euthanized or die outside of the testing period, carcasses are placed in designated facility refrigerator and VS is notified.

Testing Procedures

Survival Method:

- Identify which mouse/rat sentinel is to be monitored and ear notch the left pinna.
- Document the date of sample collection on the cage card.
- Pelt and perineum swabs collection.
  - Using a sterile, pink sticky swab, thoroughly swab the fur against the direction of the hair coat of the animal. It is important to swab around the face, back, tail base and belly for the best results. Sample both animals.
  - Insert the swab halfway into a labeled sterile tube, close the tube lid against the swab shaft and pull down on the swab shaft to break the shaft. The tip end will fall into the tube, and the tube can be capped.
- Fecal pellet collection
  - If multiple animals of the same health status are being evaluated, up to 10 fresh fecal pellets can be pooled and tested as one sample. If collecting fecal pellets from multiple cages, gloves should be changed, and forceps replaced between animals to prevent cross-contamination. Alternatively, forceps can be wiped clean and immersed in diluted bleach (10%) solution for 10 minutes prior to reuse.
- Blood collection for serology

- **Saphenous bleed** – For this bleed, two people are required. The first person must restrain the rodent and extend one rear limb. The second person will pluck fur over blood vessel and apply thin layer of topical lubricant. Using a lancet, the second person will puncture the saphenous vein and collect blood using a HemaTip™. After collection, apply gauze and hold with firm pressure until bleeding has stopped, then return the animal to its cage.
- **Tail bleed (Rats)** – For this bleed, two people are required, or one person and a restrainer is used. Restrain the rodent, then locate the vein and gently puncture the blood vessel using a lancet or needle. Place the HemaTip™ on the blood drop to saturation. After collection, apply gauze and hold with firm pressure until bleeding has stopped, then return the animal to its cage.
- **Submandibular bleed** – For this bleed only one person is required. Restrain the rodent by grasping the loose skin over the shoulders and behind the ears; the skin should be taut over the mandible. Visualize submandibular vein (at the "whirl" of the cheek). Puncture the vein with a lancet or needle and Saturate the HemaTip™. After collection, apply gauze and hold with firm pressure until bleeding has stopped, then return the animal to its cage.

Euthanasia Method:

- Place the sentinel animals in a CO_2 chamber (approximately 3-5 minutes) or observe for final breath before removing animal from the chamber.
- Collect blood from both sentinel animals via cardiac puncture for serology using a 1mL syringe.
  - Apply blood sample to HemaTip™ microsampler or EZ-Spot® Cards
  - Submit the sample from the mouse that is not ear notched, and retain the second sample in house for confirmatory testing, if needed
  - Once all the initial results have been deemed negative, discard the second hold sample
  - Pelt and perineum swabs collection.
    - Using a sterile, pink sticky swab, thoroughly swab the fur against the direction of the hair coat of the animal. It is important to swab around the face, back, tail base and belly for the best results. Sample both animals.
    - Insert the swab halfway into a labeled sterile tube, close the tube lid against the swab shaft and pull down on the swab shaft to break the shaft. The tip end will fall into the tube, and the tube can be capped.
  - Fecal pellet collection
    - If multiple animals of the same health status are being evaluated, up to 10 fresh fecal pellets can be pooled and tested as one sample. If collecting fecal pellets from multiple cages, gloves should be changed, and forceps replaced between animals to prevent cross-contamination. Alternatively, forceps can be wiped clean and immersed in diluted bleach (10%) solution for 10 minutes prior to reuse.

  - Fresh fecal pellets may also be collected directly from the large intestine during necropsy.
- Perform a gross necropsy, document any abnormal findings; abnormal findings may be processed at veterinarian's discretion.

Sentinel Placement:

- Sentinel coordinator must be notified in advance of any sentinel cages being moved to different rack or room in the facility.
- Sentinels should be moved along with their assigned colonies
- All sentinels must be placed on the bottom row far left or right of a rack.
- All occupied rodent holding rooms should have sentinel animals unless an exception is granted by the Director or Associate Director (often for short term studies less than two months duration).

Ordering and Receiving of Sentinel Animals:

- The sentinel coordinators are responsible for ordering and placing sentinel animals.
- ACS are responsible for unpacking the animal orders, though shipping coordinators can assist as needed.
- All animal orders are placed through the ACFC portal.
- Sentinel rodents must be ordered as Specific Pathogen Free (SPF) or Elite and from a Rutgers University approved commercial vendor.
- In ACFC, specify the commercial vendor, strain, age, sex, and number of sentinel animals required. Generally female CD-1 mice and CD rats from 4-8 weeks are ordered for sentinel use.
- Upon arrival, sentinel mice and rats are housed two per cage and placed in the appropriate room/rack. All sentinel cages should receive either a water bottle or hydrogel pack for the first two weeks of housing.
- Sentinel colored cage cards should be filled out by sentinel coordinators and placed on the cages. The back of the cards provide space for recording cage changes.

NOTE: Male sentinel animals may be requested by the PI based on their study needs.

Positive Test Results:

- The Attending Veterinarian and VS must be notified as soon as possible in the event of any positive sentinel test results for an excluded pathogen.
- While subsequent steps vary by facility, all positive test results initially require resubmission of that test sample(s) to confirm positive results. See SOP 7.13 Infectious Disease Outbreak Management.

Archiving Test Results:

- All results are archived and made available through the Laboratory Testing Management (LTM™) Software through Charles River Laboratories.
- Results are also accessible through Microsoft SharePoint for VS.

Charles River Serology Profiles

- Mouse Profile
  - https://www.criver.com/sites/default/files/resource-files/mouse-serology-profiles.pdf
- Rat Profile
  - https://www.criver.com/sites/default/files/resources/RatSerologyProfiles.pdf

REFERENCES:

SOP 7.13 Infectious Disease Outbreak Management