

**STANDARD OPERATING PROCEDURES**  
**DIVISION OF COMPARATIVE MEDICINE**  
**UNIVERSITY OF SOUTH FLORIDA**

SOP#: 410.19

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**TITLE:** Sentinel Rodent Health Surveillance  
**SCOPE:** All Animal Care Personnel  
**RESPONSIBILITY:** Veterinarians, All Animal Program Personnel  
**PURPOSE:** To Establish the Proper Guidelines for Monitoring Health Status of Rodent Populations Utilizing Sentinel Rodents

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**I. PURPOSE**

1. To define the microbial status of rodent colonies, surveillance is conducted for sub-clinical, clinical diseases and opportunistic agents that could jeopardize the validity and reproducibility of research data, complicating its interpretation.

**II. RESPONSIBILITY**

1. The veterinarians oversee all aspects of animal health and are assisted by all program staff.
2. The Assistant Director is responsible for ensuring that all practices are implemented by Facility Managers.
3. The Facility Manager is responsible for ensuring that all technical and animal care staff are adequately trained and experienced in sentinel rodent health procedures.
4. The Assistant Director is responsible for coordinating these sentinel rodent health procedures, submitting samples for evaluation, recording results, and reporting findings to the Director or designee.

**III. PROCEDURES**

1. **Sentinel rodents are procured from an approved commercial vendor's SPF/VAF colony.**
2. Semiannually, immunologically mature (4-5 weeks of age) **sentinels of like species are placed in each conventional rodent room**, for an exposure interval of **at least 10 weeks**. Receipt of sentinels should be coordinated to coincide with cage change-outs so that sentinels are exposed to dirty bedding upon arrival. Sentinels are housed as pairs, tested after a >10 week exposure at intervals described below depending on infectious agent excluded and facility location, then euthanized and replaced semiannually.
3. **Isolation housing** rooms located in the Stabile Research Building (SRB) will not have dirty bedding sentinels placed, but will be tested using PCR of pooled fecal samples and/or air handling unit/rack exhaust plenums. Fecal PCR will be from a representative sample of the current inventory equal to 10 percent of the population within each isolation room.
4. **Two sentinel mice or two sentinel rats are housed in individual microisolator cages and are placed on each rack with like species.** The sentinel cage is to be placed on the last right cage of the last row of a rack. One of each paired sentinels is made individually identifiable prior to

placement and used in all initial assessments (e.g., two quarterly murine assessments), with the identifier noted on the cage card. Racks housing mice on both sides will have 2 sentinel cages, 1 cage located on each side of the rack. In addition to labeling racks with the rack number, racks housing mice on both sides must be labeled to identify one side as A and the opposite side as B.

5. Once sentinels are placed, each cage is **labeled with a navy blue cage card** with a sentinel label affixed that includes the sentinel's location in the facility (room, rack, rack-side), animal information (date of birth [DOB], sex, date received, date of placement [DOP], source), and "PI & IACUC # represented."

**SENTINEL**      Miedel      IACUC #\_(xxxx)  
Species: \_\_\_\_\_ Sex: \_\_\_\_ DOB: \_\_\_\_\_ Source: \_\_\_\_\_  
Date Rec'd: \_\_\_\_\_ Date of Placement: \_\_\_\_\_  
Room: \_\_\_\_\_ Rack: \_\_\_\_\_ Side: A B Cubical: \_\_\_\_\_  
Original / Replacement ID#: \_\_\_\_\_ Tattoo/ID: \_\_\_\_\_  
PI & IACUC# Represented: \_\_\_\_\_  
\_\_\_\_\_  
Date Exposed: \_\_\_\_\_

6. **Each sentinel cage/sample is identified** by a unique code, which indicates the room number (using the last 2 digits of the room number), rack number proceeded by R (racks are numbered consecutively starting on the left with #1 when standing in the doorway facing into the room and counting clockwise around the room for racks placed along the walls, or numbered from front to rear of the room when racks are arranged in a single row), and location of the sentinel on the rack (side A = mouse sentinel cage A, and side B = mouse sentinel cage B). For example, "**43R3B**" denotes room 1343, **Rack #3**, mouse sentinel cage **B**.
7. The individually identified sentinel in each pair is used for initial assessments (i.e., first two quarterly murine assessments); either sentinel may be used when additional testing is required, as directed by the veterinarian.
8. **Progress Notes are generated when sentinels are placed in each room.** These notes should identify the animal by its unique code and include the DOB and DOP to differentiate sentinel replacements. Entries should include date of initial exposure, date of last exposure (if rack is emptied), date of re-exposure (if rack is put in use again), any sentinel movement (moved to another rack when consolidating racks, or to another room when entire colonies/racks relocate), dates and types of testing conducted, any retesting (of sentinel or colony representatives) and euthanasia.
9. **If sentinels are relocated** to another room/rack/side with the group of animals they represent, the new location/information is recorded on the cage card and in the **Progress Notes** with the date of relocation (e.g., 8/9/11 relocated to 58R1A) so that the sentinel can be traced to all its previous locations.
10. To maximize exposure to potential infectious agents, **upon arrival all sentinels are housed on soiled bedding** obtained from other cages on the same rack/side (e.g., sentinel cage A is exposed to the dirty bedding of animals housed in cages on side A, sentinel cage B is exposed to the dirty bedding of animals housed in cages on side B of the rack). See **SOP #402** for details.
11. Sentinels will continue to be exposed to dirty bedding according to the cage change-out schedule of the animals they represent (e.g., sentinels for single-housed rodents are changed/exposed once weekly, sentinels for multiple housed rodents are changed/exposed twice weekly, sentinels for rodents housed in ventilated cages are change/exposed every two weeks).

12. When mice are housed in ventilated caging systems, the interior airways of the air handling unit/rack exhaust plenums will be sampled for PCR testing.
13. **Veterinarians conduct sentinel health evaluations assisted by the animal care staff.** If test results are positive, the veterinarian will develop a plan for additional testing to determine the extent of the infestation/infection and for eradication of the agent if warranted.
  - a. **Sentinels are evaluated serologically** by phlebotomy of a sentinel from each cage and submitted using an Opti-Spot card.
  - b. **Sentinels are evaluated for fur mites** by PCR by pooling up to 10 cage/pelt swabs collected from the sentinels within the room. Alternatively, pooling can be accomplished by using a single swab to sample up to 10 sentinel cages per room. When using a single swab dirty caging will be sampled after sentinels have been removed to clean caging to prevent cross-contamination.
  - c. **Sentinels are evaluated for pinworm** by PCR by pooling up to 10 fecal pellets collected from the sentinel cages within the room. By collecting a single pellet from each sentinel cage, up to 10 cages from within the same room can be pooled for testing.
  - d. **Sentinels are evaluated for Helicobacter and MNV** by PCR testing of feces during the months of May and November. The fecal samples collected for pinworm PCR can also be tested for Helicobacter and MNV; consequently, no additional sample collection is necessary.
  - e. Sentinels remain in the cage for approximately 6 months until all testing results are received by an Assistant Director. The Assistant Director will notify the Facility Manager when all results are negative and sentinels should be euthanized and replaced.

14. **Sentinel rodents are tested for the following agents:**

AGENT	SAMPLE	TEST	FEB	MAY	AUG	NOV
<b>Mouse</b>						
MHV	serum spot	MFIA	x	x	x	x
MPV 1-5	serum spot	MFIA	x	x	x	x
MVM	serum spot	MFIA	x	x	x	x
NS1	serum spot	MFIA	x	x	x	x
TMEV	serum spot	MFIA	x	x	x	x
EDIM	serum spot	MFIA	x	x	x	x
Fur mites ( <i>Myocoptes, Myobia, Radfordia</i> )	pooled pelt/cage	PCR	x	x	x	x
Pinworms ( <i>Aspicularis, Syphacia</i> )	pooled feces	PCR	x	x	x	x
<i>Helicobacter</i>	pooled feces	PCR		x*		x*
MNV	serum spot	MFIA		x*		x*
Sendai	serum spot	MFIA				x
<i>Mycoplasma pulmonis</i>	serum spot	MFIA				x
PVM	serum spot	MFIA				x
Reo3	serum spot	MFIA				x
LCMV	serum spot	MFIA				x
Ectromelia	serum spot	MFIA				x
MAV1	serum spot	MFIA				x
MAV2	serum spot	MFIA				x
Polyomavirus	serum spot	MFIA				x
AGENT	SAMPLE	TEST	FEB	MAY	AUG	NOV
<b>Rat</b>						

\*Additional agents excluded at the SRB, ALZ, BPB, and IDR facilities

RCV	serum spot	MFIA	x	x	x	x
Parvo (NS1, RPV, RMV, KRV, H-1)	serum spot	MFIA	x	x	x	x
Fur mites ( <i>Myocoptes</i> , <i>Myobia</i> , <i>Radfordia</i> )	pooled pelt/cage	PCR	x	x	x	x
Pinworms ( <i>Aspiculuris</i> , <i>Syphacia</i> )	pooled feces	PCR	x	x	x	x
RTV	serum spot	MFIA		x		x
Sendai	serum spot	MFIA				x
PVM	serum spot	MFIA				x
<i>Mycoplasma pulmonis</i>	serum spot	MFIA				x

**Equipment surfaces in all mouse housing rooms** (i.e., interior airways of the air handling unit/rack exhaust plenums) are PCR tested for ***Corynebacterium bovis***, monthly.

At the discretion of the veterinarian, specimens collected from equipment each month may be pieces of filter paper attached to the exhaust prefilter in the air handling unit (i.e., filter-clips), or alternatively, sterile FLOQ swabs used to trace a circular pattern inside the exhaust plenum of the rack for three circumferences while rolling the swab tip.

**Immunodeficient mice in isolation housing** and **mice housed in the mouse models core** are tested for the following agents using pooled fecal or pelt/cage sampling:

AGENT	SAMPLE	TEST	FEB	MAY	AUG	NOV
<b>Mouse</b>						
MHV	pooled feces	PCR	x	x	x	x
MPV 1-5	pooled feces	PCR	x	x	x	x
MVM	pooled feces	PCR	x	x	x	x
NS1	pooled feces	PCR	x	x	x	x
TMEV	pooled feces	PCR	x	x	x	x
EDIM	pooled feces	PCR	x	x	x	x
Fur mites ( <i>Myocoptes</i> , <i>Myobia</i> , <i>Radfordia</i> )	pooled pelt/cage	PCR	x	x	x	x
Pinworms ( <i>Aspiculuris</i> , <i>Syphacia</i> )	pooled feces	PCR	x	x	x	x
<i>Corynebacterium bovis</i>	pooled feces	PCR	x	x	x	x
<i>Helicobacter</i>	pooled feces	PCR	x	x	x	x
MNV	pooled feces	PCR	x	x	x	x
<i>Corynebacterium kutscheri</i>	pooled feces	PCR	x	x	x	x
<i>Klebsiella oxytoca</i>	pooled feces	PCR	x	x	x	x
<i>Klebsiella pneumoniae</i>	pooled feces	PCR	x	x	x	x
<i>Pseudomonas aeruginosa</i>	pooled feces	PCR	x	x	x	x
<i>Pasteurella pneumotropica</i> biotype Jawetz	pooled feces	PCR	x	x	x	x
<i>Pasteurella pneumotropica</i> biotype Heyl	pooled feces	PCR	x	x	x	x
<i>Pneumocystis</i> spp.	pooled feces	PCR	x	x	x	x
<i>Proteus mirabilis</i>	pooled feces	PCR	x	x	x	x
<i>Staphylococcus xylosus</i>	pooled feces	PCR	x	x	x	x
<i>Staphylococcus aureus</i>	pooled feces	PCR	x	x	x	x

15. Sample collection methods can be found at the following links:

- a. Serum spot - <https://www.idexxbioresearch.eu/optispot-sample-collection-guidelines/?rq=sample%20collection>
- b. Fur mite PCR - <https://www.idexxbioresearch.eu/new-page-3>

c. **Fecal pellet PCR** - <https://www.idexxbioresearch.eu/fecal-pellet-sample-collection-sop>

16. Colony representative animals can be used to characterize the microbial status of rodent production colonies at each facility.
17. PIs may submit retired breeders and/or immunologically mature animals that are representative of the health history of their particular colony.
18. The findings concerning each sentinel evaluation are recorded by the Assistant Director and reported to the Director or designee for interpretation and/or resolution as needed.
19. Additional health evaluations may be conducted upon request from the research staff at the discretion of the veterinarians or in response to suspect exposure to infectious agents. Additional health evaluations may involve the use of additional sentinel animals and/or colony representatives.
20. A general rule of thumb is that at least the sentinel and >5 animals per rack side (from 5 separate microisolator cages) are retested in response to a positive serological test. This is a crude extrapolation of historical practices used to evaluate a population housed in a common air space and represents the minimum sample size necessary to achieve a 95% confidence that the presence of the agent will be detected in the animals tested, assuming transmission at a 50% infectivity rate. However, many factors determine just how a positive or suspect result is responded to and reevaluated including the biology of the specific agent, the significance of the disease in question, the impact on the research being conducted on these animals, and the value of resident colonies within the facilities, all of which play a role in determining reevaluation, containment, and eradication procedures.
21. Staff veterinarians and technical staff report sentinel rodent health evaluation findings and submit samples for diagnostic laboratory evaluations on an electronic version of the **Rodent Health Evaluation** form to the Assistant Director.
22. Results of sentinel evaluations are maintained by the Assistant Director.
23. New sentinels should be requested to allow adequate time for an appropriate exposure interval of >10 weeks.
24. If one of the pair-housed sentinels dies during the period of soiled bedding exposure, the veterinarian is consulted. **Sentinel rodents may be single housed until the next evaluation cycle.** If the rack which the sentinel represents is vacated the veterinarian will be consulted.
25. Immunodeficient mice are susceptible to opportunistic and commensal bacteria, transmission of which may occur by direct contact, via fomites including gloved hands, or via cell lines. The presence of opportunistic bacteria can be verified by PCR testing of animals (i.e., skin swabs) or the environment (i.e., IVC exhaust plenums). Husbandry procedures in accordance with SOP 413 entitled "Isolation Rodent Husbandry and Use" must be adhered to when handling immunodeficient mice.
26. When presence of non-tolerated opportunistic bacteria is confirmed by PCR testing, measures must be taken to contain and prevent further dissemination of the agent (see item 20, above, regarding factors used to determine just how a positive or suspect result is responded to and/or reevaluated).

27. When presence of an excluded or non-tolerated agent is confirmed by testing, measures taken may include but are not limited to (a) depopulation of the affected animals, (b) rederivation of affected colonies, (c) decontamination of racks, trolleys and equipment in the affected room, (d) decontamination of the room using vaporized hydrogen peroxide in accordance with **SOP 1016 Hydrogen Peroxide Vapor Decontamination** and **SOP 1162 Bioquell Z-2 Hydrogen Peroxide Vapor Generator System**, and (e) follow up retesting of the room and occupants.

## 28. IDEXX Panels, Schedules and Agents

### a. Sentinel Mouse Panels

#### USF Sentinel Mouse Panel 1 (Feb/Aug all facilities)

- Optispot: MHV, MPV1-5, MVM, NS1, TMEV, EDIM
- Fecal: Pinworms (*Aspicularis*, *Syphacia*)
- Pelt/cage: Fur mites (*Mycoptes*, *Myobia*, *Radfordia*)

#### USF Sentinel Mouse Panel 2 (May all facilities)

- Optispot: MHV, MPV1-5, MVM, NS1, TMEV, EDIM, MNV
- Fecal: Pinworms (*Aspicularis*, *Syphacia*), Helicobacter
- Pelt/cage: Fur mites (*Mycoptes*, *Myobia*, *Radfordia*)

#### USF Sentinel Mouse Panel 4 (Nov all facilities)

- Optispot: MHV, MPV1-5, MVM, NS1, TMEV, EDIM, Sendai, Mycoplasma pulmonis, PVM, Reo3, LCMV, Ectromelia, MAV1, MAV2, Polyomavirus, MNV
- Fecal: Pinworms (*Aspicularis*, *Syphacia*), Helicobacter
- Pelt/cage: Fur mites (*Mycoptes*, *Myobia*, *Radfordia*)

### b. Sentinel Rat Panels

#### USF Sentinel Rat Panel 1 (Feb & Aug)

- Optispot: RCV, Parvo (NS1, RPV, RMV, KRV, H-1),
- Fecal: Pinworms (*Aspicularis*, *Syphacia*)
- Pelt/cage: Fur mites (*Mycoptes*, *Myobia*, *Radfordia*)

#### USF Sentinel Rat Panel 2 (May)

- Optispot: RCV, Parvo (NS1, RPV, RMV, KRV, H-1), RTV
- Fecal: Pinworms (*Aspicularis*, *Syphacia*)
- Pelt/cage: Fur mites (*Mycoptes*, *Myobia*, *Radfordia*)

#### USF Sentinel Rat Panel 3 (Nov)

- Optispot: RCV, Parvo (NS1, RPV, RMV, KRV, H-1), RTV, Sendai, PVM, Mycoplasma pulmonis
- Fecal: Pinworms (*Aspicularis*, *Syphacia*)
- Pelt/cage: Fur mites (*Mycoptes*, *Myobia*, *Radfordia*)

### c. Custom PCR Panels

#### USF Isolation Mouse Panel (Feb, May, Aug, Nov)

- Pelt/cage: Fur mites (*Mycoptes*, *Myobia*, *Radfordia*)
- Fecal: MHV, MPV 1-5, MVM, NS1, TMEV, EDIM, Pinworms (*Aspicularis*, *Syphacia*), *Corynebacterium bovis*, Helicobacter, MNV, *Corynebacterium kutscheri*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Pasteurella pneumotropica* biotype Jawetz, *Pasteurella pneumotropica* biotype Heyl, *Pneumocystis* spp., *Proteus mirabilis*, *Staphylococcus xylosum*, *Staphylococcus aureus*.

Approved:

Date: