

# STANDARD OPERATING PROCEDURES

DIVISION OF COMPARATIVE MEDICINE  
UNIVERSITY OF SOUTH FLORIDA

SOP#: 411.17

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<b>TITLE:</b>	<b>Rodent Quarantine</b>
<b>SCOPE:</b>	All Animal Care Personnel
<b>RESPONSIBILITY:</b>	Veterinarians, All Animal Program Personnel
<b>PURPOSE:</b>	To Establish the Proper Guidelines for the Quarantine of Rodents

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## I. PURPOSE

1. To prevent the introduction of rodent pathogens including environmental opportunistic agents into established rodent colonies.

## II. RESPONSIBILITY

1. The veterinarians oversee all aspects of animal health, and are assisted by all program staff.
2. The Assistant Directors are responsible for ensuring that Facility Managers implement all practices.
3. The Facility Manager is responsible for ensuring that all technical and animal care staff are adequately trained and experienced in rodent quarantine procedures.

## III. PROCEDURES

1. Rodents from the **SPF/VAF colonies of approved commercial animal vendors are housed in facilities without a period of quarantine.** The Fiscal & Business Specialist is responsible for ensuring that current health statuses of the approved vendor colonies from which requested animals originate are SPF/VAF for infectious agents on the exclusion list. All animals should be acclimated a minimum of seven days prior to use.
2. Rodents from non-approved sources including other commercial vendors and from academic institutions, **regardless of written health assurances, are quarantined, treated and evaluated** as described below.
3. Access to quarantine rooms/facilities is limited to **essential personnel**. Research personnel are not allowed in quarantine areas unless escorted by a veterinarian or designee.
4. **A technician is assigned to each group of animals arriving into quarantine and is responsible for:** ensuring the procedures outlined in the quarantine schedule, performance of health evaluations, interpretation of test results, and when all is successfully completed, the released of animals from quarantine. Technicians are assigned by the facility manager or designated supervisor and assisted by other assigned animal care staff at each facility in the collection of samples.
5. Just **before entering quarantine, personnel don the appropriate protective clothing.** Dedicated quarantine scrubs and shoes, disposable gown, shoe-covers, bouffant cap, and gloves, or Tyvek® coverall, bouffant cap if Tyvek® hood is not worn, and gloves are

required within the centralized quarantine facility located in the Stabile Research Building. At a minimum, disposable gown, Tyvek® sleeves, gloves, and shoe covers, are required at all other facilities. All cages must be opened in a certified laminar flow changing station or biosafety cabinet.

6. **All disposable protective clothing is removed** after the completion of duties and disposed of in a biohazard waste container upon exiting quarantine. Prior to exiting quarantine, after removing and disposing of PPE, step on the antimicrobial adhesive floor mat positioned just inside the ante room. Hands are sanitized immediately upon exiting.
7. **Animals quarantined at the Stabile Research Building (SRB)** are received into quarantine room 20093 or 20092, depending on the health status of the arriving animals, for initial characterizations and treatments. Quarantined mice may move from 20093 into the middle quarantine housing room 20092 only when approved by a director or a veterinarian. Only SPF/VAF characterized, progeny, experimental, or surrogate colony mice are housed in the inner-most (i.e., clean) quarantine room 20094.
8. **Quarantined animals** are housed in either a ventilated cage/rack system or autoclavable microisolators, in a room separate from non-quarantined animals that is **serviced last**, by a designated technician. Ventilated caging is **changed once every two weeks** to minimize potential contact with possible shedding of infectious agent(s) harbored by quarantined rodents while defining the microbial status of such rodents.
9. **Caging equipment and water bottles are autoclaved in and out of the quarantine room.** Ensure all equipment is properly autoclaved by referring to procedures for autoclave sterilization (**SOP #1002**), sterilization (**SOP #1006**), and Verify indicators (**SOP #1007**) for proper sterilization techniques.
10. Soiled microisolator **caging is autoclaved as a unit prior to transferring to cage-wash for sanitation.** To prepare soiled caging for autoclaving:
  - a. Remove microisolator top, empty feed from wire rack and the contents of the water bottle onto the cage bedding, and either replace the uncapped water bottle in the microisolator bottom or cap the water bottle and replace in the wire rack.
  - b. **Place a Verify® Integrator strip in a representative cage for each batch of cages to be autoclaved in a decontamination cycle** so that it can be readily observed and replace the microisolator top. Do not clamp IVC microisolator tops.
  - c. **When an autoclave is not located within the quarantine room:**
    1. Place caging unit in an autoclavable biohazard bag.
    2. Seal bag (e.g., tape or twist tie), and place autoclave indicator tape on outside of bag. Note in which bagged cage the Verify® Integrator strip has been placed.
    3. Spray outside of bag with Oxivir Tb® and allow for appropriate contact time for disinfection (i.e., 5 minutes) prior to removing from the room.
    4. Transport bag directly to autoclave.
    5. Autoclave sterilization is performed in accordance with **SOP #1006** and monitored in accordance with **SOP #1002**.
11. **Work surfaces are decontaminated** with Oxivir Tb® before and after use.
12. **Standard quarantine practices** include:
  - a. Treatment of all animals for endoparasites (e.g., pinworms) by providing Harlan Teklad TD 01432 (a pelleted rodent diet containing **Fenbendazole**) *ad libitum* upon arrival through week four.

- b. Treatment for ectoparasites (e.g., fur mites) and nematode endoparasites by the application of **Ivermectin** (10 mg/ml) at a dose of 1 $\mu$ l/5grams of body weight topically between the animals scapulae on **days 1 and 10** of quarantine. The micropipette tip application of Ivermectin should be against the grain of hair growth, directly on the skin, while the animal is restrained.
- c. **Fur Mite PCR samples collected during weeks 1 and 3 for all rodents and tested by PCR** for *Mycoptes*, *Myobia*, and *Radfordia* (two options are available for testing for fur mites):
  1. **Pelt swab method:** Using a dry flocked **swab**, swab the head, rump, and inguinal area of one or more animals in the cage.
  2. **Cage swab method:** Using a dry flocked swab, swab the inside perimeter of a soiled cage at the level of the bedding. Up to 10 cages housing the same imported cohort of animals can be swabbed using the same swab.
  3. Fur mite sample collection procedures can be found at: <https://www.idexxbioresearch.eu/new-page-3>
- d. **Fecal Pellet PCR:**
  1. For **all mice**, collect and submit fecal pellets upon arrival to **test for *Helicobacter*, MNV and *Corynebacterium bovis* by PCR**. Animals testing positive will be foster rederived and forgo any additional standard quarantine testing. Progeny will be tested as described in Item #17 below.
  2. For **all rodents, collect fecal pellets during weeks 1 and 3**. Week 1 fecal pellets are labeled and stored refrigerated until pooled together with the week 3 pellets.
    - a. All mice are evaluated for evidence of MHV, MVM, MPV (MPV1-5), TMEV, EDIM, MNV, *Helicobacter* and *Corynebacterium bovis* and pinworms (*Syphacia* spp. and *Aspicularis tetraptera*).
  3. Fecal pellet sample collection procedures can be found at: <https://www.idexxbioresearch.eu/fecal-pellet-sample-collection-sop>
- e. **OptiSpot™ serology:**
  1. Test **blood collected during week 3** for evidence of antibodies indicating prior exposure to infectious agents on the exclusion list.
  2. **All mice** are evaluated serologically for Parainfluenza virus Type I (**Sendai**), Coronavirus (**MHV**), ***Mycoplasma pulmonis***, Parvovirus (**MPV 1-5, NS1, MVM**), Rotavirus (**EDIM**), Poliovirus (**TMEV**), and Calicivirus (**MNV**), Paramyxovirus (**PVM**), Reovirus (**Reo3**), Lymphocytic Choriomeningitis (**LCMV**), Orthopoxvirus (**Ectromelia**), Adenovirus (**MAV1, MAV2**), and Polyomavirus.
  3. **Rats** are evaluated for Parainfluenza virus Type I (Sendai), Coronavirus (RCV/SDAV), *Mycoplasma pulmonis*, Parvovirus (NS1, RPV, RMV, KRV, H1), Rat Theilovirus (RTV), and Paramyxovirus (PVM).
  4. OptiSpot™ sample collection procedures can be found at: <https://www.idexxbioresearch.eu/optispot-sample-collection-guidelines/?rq=sample%20collection>

13. **PI's are notified** when animals test positive for *Helicobacter*, MNV or *Corynebacterium bovis* and informed of procedures to eliminate these agents.

14. **Ideally, animals in each microisolator are evaluated by PCR and by serological tests for excluded agents.** Technicians collect specimens for the evaluation of quarantined animals according to the schedule below. **Representative animals are evaluated when large shipments are received.** When representative animals are evaluated, different animals should be selected for subsequent evaluations. If test results are positive the veterinarian/facility manager will discuss with the PI and develop a plan that will ensure delivery of the line/genotype free of evidence of exposure to agents on the exclusion list.
15. PIs are notified when animals test positive for excluded agents and informed of procedures to eliminate these agents.

	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	Mouse Agents Tested
<b>All Rodents</b>	<ul style="list-style-type: none"> <li>Collect cage swab, label and store</li> <li>Collect feces, label and store</li> <li>Submit an additional separate fecal sample for <b>Helicobacter, MNV, and C. bovis</b> PCR upon arrival</li> <li>Initiate Fenbendazole diet</li> <li>Ivermectin on day 1</li> </ul>	<ul style="list-style-type: none"> <li>Ivermectin on day 10</li> </ul>	<ul style="list-style-type: none"> <li>Submit Opti-Spot blood for quarantine serology panel</li> <li>Collect cage swab, pool with week 1 cage swab, label, and submit</li> <li>Collect feces, pool with week 1 feces, label, and submit</li> </ul>	<p><b>All mice</b></p> <ul style="list-style-type: none"> <li><b>Serology:</b> MHV, MVM, NS1, MPV (MPV1-5), MNV, TMEV, EDIM, Sendai, <i>Mycoplasma pulmonis</i>, PVM, Reo3, LCMV, Ectromelia, MAV1, MAV2, and Polyomavirus</li> <li><b>Fecal PCR:</b> MHV, MVM, MPV (MPV1-5), TMEV, EDIM, &amp; Pinworms (<i>Syphacia obvelata</i>, &amp; <i>Aspicularis tetraptera</i>), <i>Helicobacter MNV</i>, and <i>C. Bovis</i></li> <li><b>Cage Swab PCR</b> Fur Mites (<i>Myocoptes</i>, <i>Myobia</i> &amp; <i>Radfordia</i> spp.)</li> </ul> <p><b>Environmental PCR (monthly):</b> <i>C. bovis</i></p>

16. **Litters born in quarantine**, either by foster rederivation, or by breeding approved by a director will be tested as described above. Pooled fecal and cage swab samples will be collected at weaning and again 2 weeks later, and pooled for submission. In addition, a dried blood spot sample is collected from the foster dam and submitted. Litters may be released into general housing when all results are confirmed negative.
17. **Ideally, an immunologically mature (≥ 10 wks. old) representative or sentinel (e.g., representing imported immune deficient strains) of each microisolator is evaluated serologically.** Serum, swabs, and feces from representative animals are submitted to IDEXX Research Animal Diagnostic and Investigative Laboratory for testing. The **number of animals to be evaluated** is determined based on the animal's health history, the health history of the originating facility, and the method housed, but no less than 25% of the arriving animals so as to achieve a 95% confidence that the individual results reflect the population's health status (assuming an infectivity rate of 15%).
18. **Large shipments of rodents** coming into quarantine are evaluated as described above, however, the number of animals to be evaluated will be determined on a case by case basis, and will be based on the animal's health history, the health history of the originating facility, and the method housed, but no less than 10% of arriving animals so as to achieve a 95% confidence that the individual results reflects the population's health status (assuming an infectivity rate of 30%).
19. All treatment and evaluations produced as a result of these procedures are recorded on the **Rodent Quarantine Record**.
20. The Assistant Director initiates a **Rodent Quarantine Record** for each group of animals being quarantined by completing the top portion of the form to include the Quarantine

Location, Principal Investigator, IACUC Protocol #, Phone #, Species/Strain, Origin/Institution, History of Health Status, and any special quarantine instructions as prescribed on the ***Request to Receive Animals From Another Institution*** form by a Director. This record is provided to the Facility Manager of the quarantining facility at the time the ***Animal Arrival Sheet*** is distributed.

21. Upon receipt of the animals at the quarantine facility the Facility Manager/assigned technician completes the ***Rodent Quarantine Record*** by recording the Assigned Technician's name, Date of Arrival, Number of Animals and Condition at Arrival. The assigned technician then notifies the PI, Fiscal & Business Specialist and Assistant Director the number of animals received into quarantine, the sex and genotype, and the condition of the animals.
22. Entries are made to the ***Rodent Quarantine Record*** as the schedule of treatments and testing are completed. At the end of the quarantine period and after all testing has been successfully completed Facility Managers and the Assistant Director interpret results of quarantined rodents and determine whether, or when to release rodents from quarantine. Animals can only be released from quarantine when the record has been signed by a director or the Facility Manager of the quarantining facility.
23. The **Facility Manager or assigned technician of the quarantining facility notifies the PI of the quarantined animals** that their release from quarantine has been approved, verifies the new housing location, and coordinates the transfer of the animals using a ***Request to Relocate Research Animals***. This information is recorded on the ***Rodent Quarantine Record***.
24. The original ***Rodent Quarantine Record*** is sent to the Assistant Director to be filed in the animal's transfer record. A copy is maintained at the quarantine facility and a copy accompanies the animals to their new housing location.
25. Request to receive animals into quarantine for the sole purpose of euthanasia and **derivation of tissues prior to release from quarantine** must be approved by a director and is based on the animal's health history, the health history of the originating facility, and the location(s) where the tissue derivations and evaluations are conducted.
26. **Request to breed animals, other than rederivation, prior to release from quarantine** must be approved by a director, and is based on the animal's health history, the health history of the originating facility, the availability of the animals, and the availability of quarantine space.
27. Additional quarantine requirements and procedures may be imposed at the discretion of the veterinarian.

#### IV. **SRB ANIMAL RELOCATION PROCEDURES**

1. Whenever mice require relocation from the SRB mouse facility to remote use areas outside of the facility (e.g., image acquisition using the multiphoton microscope in the USF Microscopy Core) , or when mice are relocated to the SRB to utilize specialized equipment/resources, procedures are used that minimize risk of exposure to potential murine pathogens.
2. In such cases, the physical transportation, relocation, use, and decontamination of mice/caging are accomplished by Comparative Medicine SRB mouse facility staff in accordance with **IACUC Principle XI** and **SOP 1015**. Mouse use in remote/common

use areas is scheduled so that no other mice are present in the area (e.g., microscopy core).

3. The relocation of mice to or from the SRB is accomplished in autoclaved commercial filtered shipping boxes that are exterior surface decontaminated with Oxivir Tb® upon arrival at the SRB/remote area (e.g., microscopy core). Each transport box will contain a single “home” microisolator cage of mice.
4. Shared equipment in common use areas are either surface decontaminated with Oxivir Tb® (e.g., multiphoton microscope stage, inhalational anesthesia nose cone) for the appropriate contact time before and after each transport box (i.e., “home” microisolator cage) of mice are used (e.g., imaged) in accordance with **SOP 1015**.
5. Shipping boxes used in the relocation are subject to exterior surface decontamination with Oxivir Tb® upon arrival/return to the SRB facility in decontamination receiving room 20070 prior to reentry into quarantine.
6. Although animal health status is presumed to remain SPF/VAF upon return to the SRB facility, as a precaution such mice are returned to the inner-most (i.e., clean) quarantine room 20094 of the SRB mouse facility after use (e.g., multiphoton microscopic imaging).
7. Whenever SPF/VAF mice require relocation to the SRB mouse facility for use in the Small Animal Models & Imaging core, or use of resources unique to the SRB (e.g., MRI image acquisition), a ***Request to Relocate Research Animals*** must be submitted to the Assistant Director. Upon approval, mice are housed in the inner-most (i.e., clean) quarantine room 20094 of the SRB mouse facility during use. Scheduling of mouse use is made when other mice are not present in the immediate area, and surfaces and common equipment are decontaminated before and after use in accordance with **SOP 1015**.

**Approved:**

**Date:**