

STANDARD OPERATING PROCEDURES
DIVISION OF COMPARATIVE MEDICINE
UNIVERSITY OF SOUTH FLORIDA

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

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.
8. Mice with ectoparasitic (e.g., fur mites) or opportunistic bacterial concerns (e.g., *Corynebacterium bovis*) should be received into “dirty quarantine” in the College of Public Health (CPH) facility first, tested and treated as directed by the veterinarians.
9. If the opportunistic infectious agent or ectoparasitic concern is merely an exporting facility’s history of detection, the mice may be imported to CPH quarantine when available for shipping, with any overlap in CPH quarantine with other quarantined mice considered an acceptable risk. If overlapping imports have an excluded infectious agent detected on or after arrival, CM will assume the costs to the cohoused importation caused by any additional testing, treatment, and quarantine housing beyond the typical quarantine procedures.
10. If the opportunistic infectious agent or ectoparasitic concern is present in the colony or housing room of the exporting program, even after apparent resolution and extramural negative testing follow ups, CPH quarantine will be considered closed after the arrival of the shipment, and will remain closed until the mice are considered SPF and released from CPH quarantine.
11. When the opportunistic agent or ectoparasitic pathogen concerns have been alleviated, mice may be moved from CPH dirty quarantine to SRB quarantine for additional characterizations and treatments if the long term housing destination excludes *Helicobacter sp.* and MNV, or alternatively may be released directly into long term housing if *Helicobacter sp.* And MNV are not excluded, at the discretion of the veterinarians.
12. SRB 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.
13. **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.
14. **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.
15. 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**.

16. **Work surfaces are decontaminated** with Oxivir Tb® before and after use.

17. **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 1µl/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. Alternatively, cages of mice may be misted with 1-2 mL of diluted ivermectin as follows:
 1. Take 1% ivermectin and dilute 10 fold by measuring 10 mL of 1% ivermectin and 90 mL of sterile water into a spray bottle. Shake well and adjust the spray bottle nozzle so that it delivers the drug in a fine mist delivering between 1 and 2 ml (1-2 mg) of the diluted ivermectin when sprayed. (The mice should be misted and not soaked).
 2. Open the cage and move the mice together so that they form a group to mist. If there are newborn pups present (**non-furred with closed eyes**), move the adults to the clean cage first, spray the adults and then move the pups to the clean cage.
- c. **Fur Mite PCR samples are collected and submitted upon arrival and during week 3 for all rodents and tested by PCR for *Mycopetes*, *Myobia*, and *Radifordia*** (two options are available for testing for fur mites):
 1. **Pelt swab method:** Using a dry flocced **swab**, swab the head, rump, and inguinal area of one or more animals in the cage.
 2. **Cage swab method:** Using a dry flocced 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 and during week 3**. Fecal samples are evaluated for evidence of MHV, MVM, MPV (MPV1-5), TMEV, EDIM, MNV, *Helicobacter* and *Corynebacterium bovis* and pinworms (*Syphacia* spp. and *Aspicularis* tetraptera).
2. For all rats, collect and submit fecal pellets upon arrival and during week 3. Fecal samples are evaluated for evidence of H1, KRV, RCV/SDAV, RMV, RPV, RTV, *Helicobacter* spp., and pinworms (*Syphacia* spp. and *Aspicularis* tetraptera). When submitting, select IDEXX "Quarantine Panel", "feces", and "Advantage Q-Basic".
3. Animals destined for facilities that exclude *Helicobacter* and/or MNV and testing positive for these agents will be foster rederived and forgo any additional standard quarantine testing. Animals testing positive for other agents will be euthanized or treated based on the agent. Progeny from cross-fostering will be tested as described in Item #17 below.
4. 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 (RPV, RMV, KRV, H1), Rat Theilovirus (RTV), LCMV, REO3, *Pneumocystis carinii*, and Paramyxovirus (PVM). When submitting, select IDEXX "Quarantine Panel", "Opti-Spot", and Advantage Q-Basic".
4. OptiSpot™ sample collection procedures can be found at:
<https://www.idexxbioresearch.eu/optispot-sample-collection-guidelines/?rq=sample%20collection>

18. **PI's are notified** when animals test positive for *excluded agents* and informed of procedures to eliminate these agents.
19. **For IDEXX Submissions on the IDEXX Webpage please name the submissions with the designators: SRBQ, PI NAME, PI protocol number, and date.**
20. **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.

21. PIs are notified when animals test positive for excluded agents and informed of procedures to eliminate these agents.

	1 st week	2 nd week	3 rd week	Mouse Agents Tested
All Rodents	<ul style="list-style-type: none"> Collect cage swab, label and submit for USF Quarantine Pelt PCR Panel. Collect feces, label, and submit for USF Quarantine Fecal PCR Panel. Initiate Fenbendazole diet Ivermectin on day 1 	<ul style="list-style-type: none"> Ivermectin on day 10 	<ul style="list-style-type: none"> Submit Opti-Spot blood for USF Quarantine Opti-Spot Panel. Collect cage swab, label, and submit for USF Quarantine Pelt PCR Panel. Collect feces, label, and submit for USF Quarantine Fecal PCR Panel. 	<p>All mice</p> <ul style="list-style-type: none"> Serology: MHV, MVM, NS1, MPV (MPV1-5), MNV*, TMEV, EDIM, Sendai, <i>Mycoplasma pulmonis</i>, PVM, Reo3, LCMV, Ectromelia, MAV1, MAV2, and Polyomavirus Fecal PCR: MHV, MVM, MPV (MPV1-5), TMEV, EDIM, & Pinworms (<i>Syphacia obvelata</i>, & <i>Aspiculuris tetraptera</i>), <i>Helicobacter</i>* MNV*, and <i>C. bovis</i> Cage Swab PCR Fur Mites (<i>Myocoptes</i>, <i>Myobia</i> & <i>Radfordia</i> spp.) <p>Environmental PCR (monthly): <i>C. bovis</i></p> <p>* not tested for or excluded at COM facility</p>

22. **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.

23. 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%).

24. **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%).

25. All treatment and evaluations produced as a result of these procedures are recorded on the **Rodent Quarantine Record.**

26. 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.

27. 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.
28. 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.
29. 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**.
30. 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.
31. 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.
32. **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.
33. 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 &/or Reassign 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: