January 12, 2026

Dear MMRRC and RRRC users,

The following procedures are used for production of live mice and rats from cryopreserved materials, maintenance of rederived live colonies and health monitoring for animals in the Mutant Mouse Resource & Resource Center (MMRRC) and Rat Resource & Research Center (RRRC) at the University of Missouri.

1. **Facilities and husbandry**. All rodents distributed by the MMRRC and RRRC are housed in rooms W112, W113, W114, and W116 barrier facilities of the Discovery Ridge vivarium at the University of Missouri. These rooms are devoted solely for MMRRC and RRRC use. All rodents are housed in sterile microisolator caging on ventilated racks supplied with irradiated LabDiet® 5008-Formulab Diet, chlorinated water in sterile bottles, and sterile bedding.

2. **Animals**. All recipients, vasectomized males and sentinel rodents are provided through in-house colonies or purchased from vendor production areas that are free of adventitious pathogens (see health monitoring program description for exclusion list). Animal orders must be specified *Helicobacter*-free (mice and rats) and MNV free (mice). These animals are currently provided by Charles River Laboratories. For each order, health reports are examined to verify that animals have been free of excluded agents.

3. **Personnel, PPE and cage change procedures**. Personnel providing animal care, colony management and cryorecovery for the MMRRC and RRRC are dedicated to the Discovery Ridge vivarium; they provide no service for any other vivarium on the University of Missouri campus. When entering MMRRC and RRRC animal rooms, all personnel don personal protective equipment which includes a head cover, mask, disposable lab gowns, and gloves. All microisolators are opened and changed using aseptic technique inside Biological Safety Cabinets that are wetted with Peroxigard™ before cage changing procedures. During any animal handling, gloved hands are kept continuously wetted with Peroxigard™.

4. **Cryorecovery procedures**. Embryo transfer surgeries are performed in a laminar flow hood (dedicated surgery hood) with sterile technique in dedicated surgical suites contained within the W113 and W116 barrier rooms.

5. **Shipping procedures.** Shipping crates (Taconic transit cages (TTC-CLR-HIGH) for rats and large mouse orders and Jax shipping containers for small mouse orders) are filled with aspen shavings (rats) or corncob bedding (mice), autoclaved and moved to barrier facilities where client animals are housed. Immediately prior to the scheduled shipping time, crates are placed in Biological Safety Cabinets that are wetted with Peroxigard™ and irradiated rodent chow, irradiated Diet Gel, and irradiated Napa Nectar gel water packs are added. Animals are then transferred to shipping crates using gloved hands that are kept continuously wetted with Peroxigard™. Taconic and Jax packing density guidelines are followed. The MU-MMRRC's preferred couriers for live animals are Validated or Optimize; Bio-trans is also acceptable, but World Courier is prohibited.

6. **Health monitoring**. Health monitoring for MMRRC and RRRC animals consists of quarterly monitoring of soiled bedding sentinels, and quarterly PCR monitoring of environmental samples for mites and pinworms. In addition, all mouse and rat female surrogates used for cryorecovery orders are monitored and health monitoring test results provided prior to shipment. The latter is an optimal means of health monitoring as it represents a direct contact form of monitoring.

   a. Agent list and monitoring technique. For one quarter each year (December), sentinels are submitted to IDEXX BioAnalytics for an Annual Sentinel Profile (see below). For the remaining three quarters

(March, June, September), sentinels are submitted to IDEXX BioAnalytics for a “Quarterly Sentinel and Rederivation Surrogate Profile.” All surrogate females submitted for monitoring also receive the latter profile. The following tests are included:

**MU MMRRC Mouse Quarterly Sentinel and Rederivation Surrogate Profile**

i. Serologic monitoring for MHV, MVM, MPV, MNV, TMEV, EDIM, Sendai, *Mycoplasma pulmonis*, PVM, REO3, LCMV, Ectromelia, MAV1, MAV2, Polyomavirus and *Pneumocystis murina.*

ii. PCR evaluation for *Helicobacter* spp. (with speciation of positives), *Mycoplasma pulmonis*, and beta-hemolytic streptococci (Groups A, B, C, G).

iii. Parasitologic evaluation* for fur mites, mesostigmatid mites, lice, flagellates including *Spironucleus muris, Giardia muris*, *Hexamastix muris, Trichomonas muris, Tritrichomonas muris*, amoeba including any unicellular organisms demonstrating amoeboid movement or cyst formations including *Entamoeba muris,* pinworms and tapeworms.

iv. Microbiologic evaluation (culture) for *Citrobacter rodentium, Klebsiella oxytoca, Klebsiella pneumoniae, Rodentibacter pneumotropicus* and *Rodentibacter heylii* (formerly *Pasteurella pneumotropica), Streptococcus pneumoniae, Salmonella* spp. and *Bordetella hinzii.*

**MU MMRRC Mouse Annual Sentinel Profile**

i. Serologic monitoring for all agents in the quarterly profile plus *Encephalitozoon cuniculi*, *Filobacterium rodentium* (CAR bacillus), *Clostridium piliforme*, MCMV, K virus, LDEV Hantaan, and MTV.

ii. PCR evaluation for all agents in the quarterly profile plus *Cryptosporidium* spp. and *Streptobacillus moniliformis*.

iii. Parasitologic evaluation* for all agents in the quarterly profile.

iv. Microbiologic evaluation (culture) for all agents in the quarterly profile plus *Corynebacterium kutscheri, Pasteurella multocida, Bordetella bronchiseptica,* and *Corynebacterium bovis*.

**MU RRRC Rat Quarterly Sentinel and Rederivation Surrogate Profile**

i Serologic monitoring for RCV/SDAV, Sendai, PVM, RPV, RMV, KRV, H-1, *Mycoplasma pulmonis*, REO3, LCMV, *Pneumocystis carinii* and RTV.

ii. PCR evaluation for *Helicobacter* spp. (with speciation of positives), *Mycoplasma pulmonis,* and beta-hemolytic streptococci (Groups A, B, C, G).

iii. Parasitologic evaluation* for: fur mites, mesostigmatid mites, lice, *Spironucleus muris, Giardia muris*, *Hexamastix muris, Trichomonas muris, Tritrichomonas muris*, amoeba including any unicellular organisms demonstrating amoeboid movement or cyst formations including *Entamoeba muris,* pinworms and tapeworms.

iv. Microbiologic evaluation for *Rodentibacter pneumotropicus* and *Rodentibacter heylii* (formerly *Pasteurella pneumotropica), Streptococcus pneumoniae* and *Salmonella* spp.

**MU RRRC Rat Annual Sentinel Profile**

i. Serologic monitoring for all agents in the quarterly profile plus MAV1, MAV2, IDIR, Hantaan, *Filobacterium rodentium* (CAR bacillus), *Clostridium piliforme,* and *Encephalitozoon cuniculi*.

ii. PCR evaluation for all agents in the quarterly profile plus *Streptobacillus moniliformis.*

iii. Parasitologic evaluation* for all agents in the quarterly profile.

iv. Microbiologic evaluation for *Bordetella bronchiseptica. Corynebacterium kutscheri, Pasteurella multocida, Rodentibacter pneumotropicus* and *Rodentibacter heylii (*formerly *Pasteurella pneumotropica), Streptococcus pneumoniae* and *Salmonella* spp.

*monitoring techniques include subgross examination of cecal contents and pelage, and examination of direct smears of cecal and jejunal contents and perianal tape impressions

b. **Quarterly environmental monitoring for mites and pinworms**. At the same time that sentinels are submitted (i.e., each quarter), 10-20 cages (depending on room cage density) of colony animals in each room are randomly chosen for mite and pinworm PCR testing. Cages are swabbed using the cage swab method described by IDEXX-BioAnalytics and one swab is used for 10 cages so that two swabs per room are obtained. Swabs are submitted to IDEXX- BioAnalytics for mite PCR testing. Similarly, fecal samples are collected from each of the chosen cages and pools of 10 fecal samples are submitted for pinworm testing.

c. **Soiled bedding sentinel program procedures**. Quarterly sentinel health monitoring is performed as follows: Sentinel animals are placed two per cage on each side of each rack in rooms W112, W113, W114 and W116. Sentinel cages are changed every week. Using a designated 1-ounce scoop, bedding from the dirtiest part of the cage is collected from each cage from the relevant rack side and placed into a new cage. Sentinels are then transferred to this cage. Sentinel animals are collected and submitted for pathogen testing every three months to IDEXX BioAnalytics.

7. **Infectious Disease Status and Biosecurity Maintenance of the University of Missouri Mutant Mouse Resource (MMRRC) and Research Center and Rat Resource and Research Center (RRRC) vivarium**

The MMRRC and RRRC vivarium is located at Discovery Ridge, a building approximately 5 miles from the main University campus and its rodent facilities. The Discovery Ridge vivarium is solely occupied by faculty, staff and students affiliated with these centers or IDEXX BioAnalytics. Access to both the building and the vivarium is restricted to authorized personnel and requires institution-issued ID badge access via card reader. Access to the vivarium requires thorough biosecurity training that includes training in traffic patterns, infectious disease prevention, use of personal protective equipment, hood procedures, etc. (SOP available on request). The vivarium consists of rooms dedicate to three basic areas:

a. Four rooms are dedicated to production of animals that are distributed by the MMRRC and RRRC. All animals that enter these rooms are either rederived into the room (two rooms have dedicated surgery suites) or purchased from approved vendors (from rooms that meet our exclusion criteria). Vendors include Charles River, Inotiv, Taconic, and The Jackson Laboratory. These rooms are locked, and entry is limited to animal care, colony management, cryorecovery, and veterinary staff. Entry can only occur if no other animal rooms in the facility or elsewhere on campus have been entered that day.

b. In addition to the four production rooms, two additional rooms are dedicated to receipt of MMRRC and RRRC animals that are submitted for embryo cryopreservation or rederivation into production rooms. Animals submitted for sperm cryopreservation do not usually enter the vivarium. Health reports from the submitting institution are carefully reviewed by the facility veterinarian to determine appropriate course of action. All animals that enter these rooms are tested on arrival for pinworms and mites by PCR at IDEXX BioAnalytics. In the past three years,

health reports from submitting institutions have revealed histories of the following agents: MPV, RPV, MNV, *Helicobacter* spp., pinworms and mites. Courses of action include:

i. Animals from facilities with a history of MPV, RPV, MNV, *Helicobacter* spp. are accepted into these rooms for rederivation or germplasm cryopreservation.

ii. Animals from facilities with a history of pinworms or mites AND from which there is convincing evidence that these agents have been eliminated, are tested on arrival for these agents.

iii. Animals from facilities with evidence of endemic infections are placed in a quarantine facility on campus until they can be tested and treated if positive.

iv. With these practices, no pinworms or mites have been detected in the past three years and no spread of MPV, RPV, MNV or *Helicobacter* spp. to sentinels or other rooms in the facility has occurred.

v. Entry into these rooms precludes entry into production rooms that day or requires showering and change of clothes.

c. One additional room is dedicated to research being performed by the MMRRC and RRRC. These projects generally use animals from the aforementioned approved vendors. Several projects are assessing the role of microbiota on animal models of disease. This may include controlled infection by *Helicobacter* spp. This infection is readily controlled by the use of ventilated racks and husbandry practices designed to prevent spread. Our faculty have studied *Helicobacter* spp. for over 20 years and at no time has spread to sentinels or other rooms in the facility occurred. Entry into these rooms precludes entry into production rooms that day.

8. **Outbreak History.** Since the MMRRC and the RRRC established the Discovery Ridge vivarium in 2008, there have been no outbreaks of naturally occurring infectious disease in these facilities. Health reports dating back to 2012 can be found at http://www.mu-mmrrc.com/Recent_Health_Report/ (MMRRC) and http://www.rrrc.us/Health_Monitoring_/ (RRRC)

In June 2014, we were notified by Taconic that our vivarium had received mice from their *Syphacia obvelata* contaminated IBU506 rooms. All affected animals received had been housed in rooms devoted to MMRRC microbiota research, and no infected animals ever entered MMRRC production or distribution rooms (W112, W113 and W114). All animals that had come from Taconic and those exposed to Taconic animals were identified and eliminated. A small colony of valuable study mice from the exposed room that had no direct contact with Taconic animals were quarantined and monitored weekly for *S. obvelata* by PCR of feces for four months. The entire facility was also checked two times over the course of the next four months. No positive animals were found. The entire facility continues to be monitored and all testing has been negative for *S. obvelata*.

Please contact us if you have questions about any of our procedures and thank you for using the MMRRC and RRRC.

Sincerely,

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