

March 16, 2020

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 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 Purina Mills 5053 PicoLab© Rodent Diet 20 or irradiated 5058 PicoLab© Mouse Diet 20, autoclaved 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 sentinel program description for list). *Helicobacter*-free and MNV-free animals must be specified on every mouse animal order. 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 working with animals don personal protective equipment which includes a head cover, mask, disposable lab gowns, and double gloves. All microisolators are opened and changed using aseptic technique inside Biological Safety Cabinets that are wetted with 10% bleach before cage changing procedures. During any animal handling, gloved hands are kept continuously wetted with 10% bleach.
- NOTICE:** Because of the severe shortage of hospital masks in the country's health care system during the current Covid-19 concern, our mask usage in MMRRC/RRRC rooms will be modified slightly for the foreseeable future. Mask usage and all other PPE requirements will continue in these rooms whenever animal cages are to be opened. However, if personnel need to enter rooms to perform routine checks where cages are not opened, they will not be required to wear masks. Thank you for your understanding.
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 barriers.
 5. **Health monitoring.** Health monitoring for MMRRC and RRRC animals consists of quarterly monitoring of sentinels as well as monitoring of female recipients used for cryorecovery. 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 (September), sentinels are submitted to IDEXX BioAnalytics for an Annual Sentinel Profile (see below). For the remaining three quarters (December, March, June), sentinels are submitted for a "Quarterly Sentinel and Rederivation Surrogate Profile." All recipient females submitted for monitoring also receive the latter profile. The following tests are included:

MU MMRRC Mouse Quarterly Sentinel and Rederivation Surrogate Profile (as of October, 2019)

- 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, *Spironucleus muris*, *Giardia muris*, large intestinal flagellates and amoeba, pinworms and tapeworms
- iv. Microbiologic evaluation (culture) for *Citrobacter rodentium*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Pasteurella pneumotropica*, *Streptococcus pneumoniae*, *Salmonella enterica*, and *Bordetella hinzii*

MU MMRRC Mouse Annual Sentinel Profile (as of October, 2019)

- 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 (as of October, 2019)

- 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*, large intestinal flagellates and amoeba, pinworms and tapeworms
- iv. Microbiologic evaluation for *Pasteurella pneumotropica*, *Streptococcus pneumoniae* and *Salmonella enterica*

MU RRRC Rat Annual Sentinel Profile (as of October, 2019)

- 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 *Streptobacillus moniliformis*
- iii. Parasitologic evaluation* for: fur mites, mesostigmatid mites, lice, *Spironucleus muris*, *Giardia muris*, large intestinal flagellates and amoeba, pinworms and tapeworms
- iv. Microbiologic evaluation for *Bordetella bronchiseptica*, *Corynebacterium kutscheri*, *Pasteurella multocida*, *Pasteurella pneumotropica*, *Streptococcus pneumoniae* and *Salmonella enterica*

*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. **Sentinel program procedures** (prior to December 1, 2012). Quarterly sentinel health monitoring (dirty bedding transfer) was performed as follows: Each mouse and rat rack within the facility had one sentinel cage housing up to two mice or two rats each. Each week, sentinel animals were exposed to approximately five cc of dirty bedding collected from cages of experimental animals. Sentinel mice were exposed to dirty bedding from up to 14 experimental cages from two rows on

the ventilated rack; the following week they were exposed up to 14 experimental cages from another two rows and so on until the entire rack of experimental animals had been sampled. Sentinel rats were exposed to dirty bedding from up to eight experimental cages from two rows on the ventilated rack; the following week they were exposed to up to dirty bedding from 8 experimental cages from another two rows and so on until the entire rack of experimental animals had been sampled.

- c. **Sentinel program procedures** (after December 1, 2012). Quarterly sentinel health monitoring is performed as follows (more information can be found in the sentinel program SOP). Sentinel animals are placed two per cage on each side of each rack in rooms W112, W113 and W114. 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. At the same time that sentinels are submitted (i.e. each quarter), 20 cages from each room are randomly chosen for mite PCR testing. Cages are swabbed using the cage swab method described by IDEXX-RADIL and one swab is used for 10 cages so that two swabs per room are obtained. Swabs are submitted to IDEXX-RADIL for mite PCR testing.

6. **Infectious Disease Status 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. Discovery Ridge is solely occupied by faculty, staff and students affiliated with these centers or IDEXX BioAnalytics. Access to both the building and vivarium require biometric fingerprint identification. 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, Envigo, 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. No pathogens on our exclusion list have been detected in the past three years.
- b. One room is 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 to determine appropriate course of action. 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 this room precludes entry into production rooms that day.
- c. Three rooms are 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. Two such models, the IL-10 knockout mouse of inflammatory bowel disease, and the *Smad3* knockout model of colon cancer require controlled infection by *Helicobacter* spp. This infection is readily controlled by the use of ventilated racks and husbandry practices designed to prevent spread. In the past three years, no spread of *Helicobacter* spp. to sentinels or other rooms in the facility has occurred. Entry into these rooms precludes entry into production rooms that day.

7. **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.

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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