

September 19, 2016

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 and W114 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 5058 PicoLab[®] Mouse Diet 20, autoclaved water in sterile bottles, and sterile bedding.
2. **Animals.** All recipients, vasectomized males and sentinel rodents are 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 with production area K64, K84 & R09 used for mice and areas K97, R04 & R08 used for rats.
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 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.
4. **Cryorecovery procedures.** Embryo transfer surgeries are performed in a laminar flow hood (dedicated surgery hood) with sterile technique in a dedicated surgical suite contained within the W113 barrier.
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 (both for cryorecovery orders and when establishing all live colonies). The latter is an optimal means of health monitoring as it represents a direct contact form of monitoring.
 - a. Agent list and monitoring technique. Quarterly sentinels and recipient females are submitted to IDEXX RADIL for a profile which includes the following tests:

Mice

- i. Serologic monitoring for Clostridium piliforme, Mycoplasma pulmonis, CAR bacillus, Ectromelia, EDIM, LCMV, LDEV, MAD1, MAD2, MCMV, MHV, MNV, MPV, MVM, Polyomavirus, PVM, REO3, Sendai, TMEV, Encephalitozoon cuniculi

- ii. PCR evaluation for *Helicobacter* spp. (with speciation of positives), *Mycoplasma pulmonis*
- 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*, *Corynebacterium kutscheri*, *Klebsiella oxytoca*, *Klebsiella pneumonia*, *Pasteurella multocida*, *Pasteurella pneumotropica*, *Salmonella enterica*, *Streptococcus pneumoniae*

Rats

- i. Serologic monitoring for *Clostridium piliforme*, *Mycoplasma pulmonis*, CAR bacillus, H1, KRV, LCMV, MAD1, PVM, RCV/SDAV, REO3, RMV, RPV, RTV, Hantaan, Sendai
- ii. PCR evaluation for *Helicobacter* spp. (with speciation of positives), *Mycoplasma pulmonis*, *Pneumocystis carinii*, *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 *Corynebacterium kutscheri*, *Klebsiella oxytoca*, *Klebsiella pneumonia*, *Pasteurella multocida*, *Pasteurella pneumotropica*, *Salmonella enterica*, *Streptococcus pneumoniae*

*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. **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 early 2011, *Klebsiella pneumoniae* was detected in sentinel rats. This was quickly traced to the vendor and did not spread to colony animals; no contaminated animals were distributed.

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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