A. General:
1. All rodents housed in UW-Madison animal facilities shall be routinely monitored for significant pathogens. Exceptions to this general policy are:
   1.1 Quarantined rodents shall be screened for pathogens according to veterinary direction based on source of animals and planned use.
   1.2 Short term studies or other study exemptions
   1.3 Rooms with animals that have been treated with or exposed to biohazardous agents (BSL3) will not be included in the normal sentinel program.
2. Routine health monitoring will consist of necropsy, serology, parasitology, +/- PCR testing on sentinel animals/samples.
   2.1 Alternatively colony animals may be used under certain circumstances under veterinary guidance.

2 Frequency: Rodent sentinels will be examined either quarterly or semi-annually in accordance with the policies of the college and as directed by its clinical lab animal veterinarian.

3 Diagnostics:
   3.1 Serologic screening for mice and rats shall annually consist of serological assessment where live rodents are transported to the diagnostic facility quarterly or twice a year. Serological profiles may alternate between “Clinical” to “Comprehensive” (RADIL) or “Assessment” and “Prevalent” panels (Charles River). Serum is collected in-house with “Prevalent” panels for Charles River collected at the SMPH and Waisman Center and all RADIL samples collected by the CPL technician for all other areas of campus. Other serological panels may be substituted or added to this schedule on agreement by the clinical lab animal veterinarian, the facility manager/investigator and the CPL pathology director.
   3.2 Should a novel infectious disease be detected in a rodent room during a “Prevalent” or “Clinical” panel a subsequent “Assessment” or “Comprehensive” panel will be submitted utilizing the back-up sera and/or sera from colony animals in the same rack and room.
   3.3 Helicobacter sp. PCR testing will be determined on agreement by the clinical lab animal veterinarian, the facility manager and investigator.
      A pooled sample of feces will be collected from each “room” of sentinels for PCR testing.
   3.4 Parasitology screening all facilities
      3.4.1 Parasitology shall routinely consist of a gross examination of the pelt for external parasites, examination of cecal contents under a dissecting microscope and anal tape sampling examined under microscope. Fecal flotation of individual
3.4 or pooled samples for intestinal parasites is done routinely at the CPL but may be done if indicated or at the discretion of the veterinarian.

3.5 Additional diagnostic tests may be ordered at the discretion of the veterinarian or as requested by the investigator.

4 Sentinel Animal Type & Quality Control:

4.1 Sentinel mice and rats shall come from approved vendors (e.g. Harlan, Taconic, CRL) and be approximately 4 weeks old and specific pathogen free at the time of placement. Other rodent species shall be between 2-6 months old and SPF at the time of placement. Outbred mice and rats will be utilized in mouse and rat rooms respectively. Sentinel animals will be procured from reputable vendors with stringent disease monitoring programs.

4.1.1 For rodent species from which serologic testing is not currently validated by diagnostic labs (e.g. Peromyscus spp.) laboratory mice and/or rats will be used as sentinels at the discretion of the veterinarian.

4.1.2 Mice and/or rat sentinels may also be used in lieu of an animal of the same genus and species (e.g. hamster, gerbil) at the discretion of the veterinarian.

4.2 Extra sentinel animals from a shipment may be used for quality control purposes. These animals will not be subjected to dirty bedding from colony cages. In conjunction with the regular sentinel monitoring for a facility, serology and parasitology may be performed on these animals to confirm the health status of sentinel animals.

5. Serology Sample Collection and Submission:

5.1. Sentinel animals shall be collected and transported to a procedure or necropsy room in a microisolator cage or other designated container for euthanasia and sample collection.

5.2. Blood samples shall typically be collected by intracardiac stick with a needle and syringe or by severing the axillary vessels with a scalpel and collecting blood with a pipette. Animals must be anesthetized or euthanized prior to blood collection, and must be euthanized at the end of the procedure.

5.3. Blood samples shall be allowed to clot, centrifuged and the serum decanted into sample vials. Serum will be diluted to diagnostic laboratories specifications.

5.3.1. Typically dilute to 1 part serum in 4 parts physiologic saline or phosphate-buffered saline, before submission or freezing.

5.3.2. Each serum sample from rats, hamsters, and guinea pigs will be split into 2 samples. Blood samples from mice in the same cage will be numbered separately in those units using multiple sentinel animals in a cage.

5.4. The serum samples shall be identified in such a way that the date of collection, room number, rack number and side of rack (if applicable) can be determined at a later date.

5.5. One of the two sentinel serum samples shall be stored frozen. The stored sample, at the discretion of the clinical veterinarian, may be used to confirm positive results, repeat indeterminate results, or replace lost samples as required. Unused banked frozen serum samples shall be held for 12 months and then destroyed.

5.6. Samples shall be sent to the diagnostic lab following their recommended procedures.
6. **PCR Fecal Sample Collection and Submission:**
   6.1. Fresh feces shall be collected from at least one mouse in each cage in a room. A pooled sample may contain up to 10 fecal pellets. Rooms having over 10 sentinel cages will require additional pooled samples.
   6.2. Samples should be collected into a sterile container.
   6.3. The fecal samples shall be identified in such a way that the date of collection and room number can be determined at a later date.
   6.4. Samples shall be sent to the diagnostic lab following their recommended procedures.

7. **Parasitology Procedures:**
   7.1. Anal tape tests shall be performed on each sentinel animal at CPL or one sentinel animal from each cage at SMPH or if directed by the clinical lab animal veterinarian.
      7.1.1. A small square of tape is applied to the anal area of the animal and then placed on the corresponding region of a microscope slide.
      7.1.2. The slide is then examined under a microscope at a minimum of 40x total magnification for parasite ova.
      7.1.3. Positive tape tests will then be identified with the corresponding room number rack number and side of rack (if applicable).
      7.1.4. Tape tests may be performed on live or recently dead animals.
   7.2. Cecal examinations shall be performed on each sentinel animal at CPL or one sentinel animal from each cage if directed by the clinical lab animal veterinarian.
      7.2.1. Once the animal is euthanized, make a midline abdominal incision and remove the cecum and directly adjacent ileum and colon (~ 1cm length).
      7.2.2. Place the cecum and intestines in a petri dish labeled with the appropriate room number and other pertinent location information.
      7.2.3. Macerate the cecum and intestines.
      7.2.4. After maceration, add warm tap water to cover the entire cecum and intestines.
      7.2.5. Cover and place the petri dish in an incubator set between 37-43 degrees Celsius for at least 10 minutes, but no longer than 2 hours.
      7.2.6. After incubation, examine the contents of the petri dish for any evidence of internal parasites under a dissecting scope at 4x and 10x total magnification.
      7.2.7. Positive samples are identified with the corresponding room number rack number and side of rack (if applicable).
   7.3. Pelt examination shall be performed on each sentinel animal at CPL or one sentinel animal from each cage if directed by the clinical lab animal veterinarian.
      7.3.1. Once blood is collected and the animal is euthanized, identify the animal carcass with room number and other pertinent location information.
      7.3.2. Examine the pelt of the animal under a dissecting scope set at a minimum of 20x total magnification. The best region to identify parasites is between the nose and ears on each lateral side and around the eyes.
      7.3.3. Pelt exams may also be done on live animals that are briefly anesthetized with isoflurane anesthesia.
      7.3.4. Positive samples are identified with the corresponding room number rack number and side of rack (if applicable).
7.4. Fecal flotations are done on each sentinel animal at CPL but may be done on pooled samples in other units by direction of the lab animal veterinarian.
7.4.1. Collect feces from the colon of the animal at necropsy or from the desired cage to be tested (about 10-15 fecal pellets for mice, 5 fecal pellets for rats).
7.4.2. Place fecal material in a coulter cup (CPL) or conical centrifuge tube (SMPH) and mix with ~ 15mL of water. Thoroughly mix the fecal material in the water, breaking it up as much as possible. SMPH - Centrifuge the mixture. Once done, pour off the liquid part of the mixture (the feces should have settled).
7.4.3. Add zinc sulfate (or other fecal floatation solution) to the tubes filling just until a meniscus forms at the top. Then place a cover slip over that meniscus and let sit for 5 min. or recentrifuge the tube.
7.4.4. After wait or centrifugation, remove the coverslip and place on a slide to be examined under a microscope for parasite ova at all powers up to 40x magnification.
7.4.5. All samples are identified with the corresponding room number rack number and side of rack (if applicable). A parasitology form is filled out (CPL) recording the result whether negative or positive. All samples from one room may be recorded on the same form.
7.4.6. The zinc sulfate must be discarded in accordance the safety department’s recommendations.

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