FLORIDA INTERNATIONAL UNIVERSITY

SOP #: 501.03

Title: SOP - Rodent Health Surveillance

Approvals:

Attending Veterinarian _____ **Date:** 6/13/2016

1. Purpose

1.1 To monitor the health status of mice and rats in an effort to prevent, detect and control the presence of specific infectious pathogens which may adversely affect animal health and/or influence research protocols.

1.2 Subclinical microbial, particularly viral infections occur frequently in conventionally maintained rodents, but also can occur on animals maintained in microisolator, individual ventilated cages. Example of infectious agents that can be subclinical but induce profound immunologic changes, or alter physiologic, pharmacologic or toxicologic responses are Sendai Virus, Kilham Rat Virus, mouse hepatitis virus, lymphocytic choriomeningitis virus and Mycoplasma pulmonis. Some agents have zoonotic potential.

2. Responsibility

2.1 The Director develops the program and identifies all which areas housing rodents are monitored areas.

2.2 The Assistant Director (AD) initiates these procedures, ensure adherence to this SOP, and ensures employees responsible for performing these procedures are trained according to this SOP.

2.3 The Animal Care Technicians (ACT) executes these procedures.

3. Definitions

3.1 Infection: Bacterial, viral and parasite pathogens, opportunist and commensals, vary considerably in pathogenicity. Most natural infections are subclinical. It is not synonymous with disease.

3.2 Zoonoses: A disease of animals (such as Hantaan) that can be transmitted to humans.

3.3 Pathogens monitored:

3.3.1 TMEV: Theiler's Murine Encephalomyelitis Virus; EDIM: Epizootic diarrhea of infant mice virus - a mouse rotavirus; PVM: Pneumonia virus of mice - a rodent pneumovirus; MNV: Murine norovirus - a mouse calicivirus; MHV: Mouse hepatitis virus. (Importance: reported to alter many experimental results); MPV: Mouse parvo virus. (Importance: interference with oncology and immunology studies); Sendai virus (Importance: alters the host response to transplantable tumors, isograft rejection is altered, changes in the phagocytic function of pulmonary macrophages. It is extremely contagious); Reo-3: respiratory enteric virus III (Importance: occasional contaminant and may interfere with research involving transplantable tumor and cell lines); LCMV: lymphocytic choriomeningitis virus (has inhibitory effect on tumor induction by various viruses, and induces abrogation of IDDM in BB rats); MCMV: mouse cytomegalovirus (Importance: can infect salivary glands and possible the pancreas); MMV (Mouse Minute Virus): (Importance: contaminant of transplantable tumors and cell lines); Ectromelia Virus (Importance: up to 100% of the animal from an experiment can die in an explosive outbreak. Some manipulations of the animals can exacerbate ectromelia virus infections); Polyoma Virus (Importance: contaminates tumor lines and other biologic materials that are passaged in mice); Sialodacryoadenitis Virus/ Rat Coronavirus (Importance: can seriously complicate studies involving the respiratory tract); Mycoplasma pulmonis (Importance: animal morbidity and mortality can interfere with long term studies, and produces lymphokine-like substances that are mitogenic for B and T lymphocytes in vitro); CARB (Cilia Associated Respiratory Bacillus): induces clinical signs similar with those of severe murine respiratory micoplasmosis; Encephalitozoon cuniculi (Importance: a protozoan which induces histological changes into the brain and kidney. Alter host responses during tumor passages in mice, and modifies natural killer cell activity); Syphacia sp.: mouse and rat pinworm. (Importance: very common endoparasites including stocks derived by cesarean section and maintained in the barrier facilities).

4. Guidelines

4.1 There are various components of the Surveillance program that corroborated will maintain a high health status of the animals housed and cared in the Animal Care Facility.

4.2 Approved Vendor Screens:

4.2.1 To prevent the introduction of adventitious agents into the animal facility, the "Approved Vendor" list is established and maintained by the ACF and provides rodent vendors that are currently evaluated and approved as procurement sources.

4.2.2 If specific animal strains need to be acquired from contaminated vendor areas and they are not readily available through other approved sources, then approval is required from the FIU Attending Veterinarian, before the delivery can be confirmed.

4.2.3 If the transfer request is approved, special arrangements must be made to ensure that these animals are isolated from approved source animals and placed in quarantine for treatment or rederivation.

4.2.4 Source of sentinel animals: are specifically purchased animals. Preferably should be of the same population as the principal population, (with the exception of immunocompromised animals, when the attending veterinarian will decide which strain to be used). The introduction of a second population as sentinels, even if it is tested and found to be free of pathogens poses a risk for contaminating the principal population. Therefore as an alternative clean ICR females from our own colony could be used as sentinels.

4.3 Sentinel Animals - Housing and Sampling Procedure

4.3.1 Placement of sentinel MICE & RATS within the designated animal holding areas

4.3.2 Provide each designated room, or rack with ventilated microisolator caging with a minimum of one sentinel cage.

4.3.3 Place a sentinel cage (containing 4-week old sentinels) on each side of double-sided (DS) racks; however, one cage may be designated per DS rack by veterinary management. Provide each rack a minimum of one cage of sentinels.

4.3.4 Each mouse sentinel cage contains ~4 week-old female ICR or CD-1 sentinel mice ordered from approved vendors with defined pathogen free status, including being Helicobacter and Murine Norovirus negative. Nude colonies are assigned heterozygous nudes as sentinels.

4.3.5 Each rat sentinel cage contains ~4 week-old female Long Evans, CD or Sprague Dawley's sentinel rats ordered from approved vendors with defined pathogen free status. Nude colonies are assigned heterozygous nudes as sentinels.

4.3.6 A minimum of two sentinels are placed in each sentinel cage,

4.3.7 Place sentinel cage(s) on the lower right or left corner of the last row of the rack and clearly label the cage as sentinel using a green card.

4.3.8 If a ventilated rack is "changed-out" (sanitization, repair, etc.) the rack identification number will be transferred to the replacement rack.

4.3.9 If a ventilated rack containing sentinel animals is considered for consolidation, the Director must first be contacted before the consolidation takes place.

4.3.10 Sentinel cage cards.

4.3.10.1 Each sentinel cage card (to include copies) will be annotated to record the room number and specific rack number.

4.3.11 When cage(s) of resident murine sentinels exist in an area that has recently completed a sentinel testing sequence, the remaining sentinel rodents are maintained in-place until the testing results are received.

4.3.12 If the test results come back negative the remaining sentinel is housed with new sentinels through the following quarter. Housing a sentinel from the preceding quarter with the new sentinels provides continuity and passes along potential pathogens from the preceding quarter. The sentinel from the preceding quarter is not tested unless indicated by the Director, but it is euthanized at the end of current quarter after all results are obtained.

4.3.13 If the test results come back positive, veterinary management will prescribe any further sample collection and testing to be performed.

4.3.14 Procedures for sentinel soiled bedding.

4.3.14.1 The laboratory animal technician changing the cages in the designated area is responsible for providing the sentinel mice with soiled bedding (from cages in the same rack as the sentinel mice) as outlined in Sentinel Soiled Bedding Procedures (SOP 502.01).

4.3.15 Procedures and schedule for testing sentinel rodents

4.3.15.1 The Senior Animal Technicians or designee are responsible for maintaining the testing schedule as outlined in Procedures for Scheduled Testing of Sentinel Rodents SOP (SOP 503.01).

4.3.16 Procedures for movement of rooms or racks of animals.

4.3.16.1 When an entire population of a room is relocated to another room, the sentinel animals remain on the rack and are moved to the new room with the population they are monitoring. A notation is made on the sentinel cage cards of the date of the relocation and the new room number. The old room number should remain on the cage card to indicate the history of the room/sentinels.

4.3.16.2 When an individual rack is relocated to another room, sentinel cages on the rack should be relocated with the rack it is monitoring. A notation is made on the sentinel cage card of the date of the relocation and the new room number, as above. The rack number should not be changed if possible. If it is

necessary to change the rack number, the old rack number should also be left on the sentinel cage card to indicate the history of the rack/sentinels.

4.3.16.3 The supervisor is responsible for the correct identification of the relocated racks.

4.3.16.4 The supervisor is responsible for notifying the AV that the racks have been relocated.

4.3.17 Closure of rooms or racks.

4.3.17.1 When a room or rack is depopulated in its entirety, the sentinel animals may be euthanized unless there is a reason for information that they may provide. If there is a necessity for collecting information from the sentinels, they will be collected and tested as necessary.

4.3.18 Procedures for consolidation of rooms or racks.

4.3.18.1 In the event that microisolator cages in rooms or racks that are housed are consolidated, an effort should be made to maintain the sentinel animals with the animals they are monitoring at least until a testing opportunity develops.

4.3.18.2 When microisolator racks are consolidated the population from one rack may be isolated on one side of the rack or on specific shelves of a rack with its sentinel cage and the side or shelves labeled accordingly. Once testing has been performed new sentinels are placed, providing that the animals on the consolidated rack are all of the same health status, one cage of sentinel

4.3.19 Procedures for setting up rooms or racks.

4.3.19.1 When a new room or rack is set up, the ACT of the assigned room notifies the supervisor that sentinels can be placed.

4.3.20 Sentinel testing schedule.

4.3.20.1 If the date of the first placement of soiled bedding is six (6) weeks prior to the scheduled quarterly testing the cage will be tested during the upcoming quarterly test period.

4.3.20.2 If the date of the first placement of soiled bedding is less than six (6) weeks from the scheduled testing the new Sentinels will be tested during the following quarter.

4.3.20.3 The supervisor records date of the first addition of soiled bedding to the new cages from the room husbandry check sheets.

4.3.21 Health checks.

4.3.21.1 The husbandry staff performs daily health checks and records the death of sentinel animals on the cage card and immediately notifies the supervisor and AV of any sentinel deaths.

4.3.22 Collection of specimens and clinical procedures

4.3.22.1 Samples collected and analyzed: main testing is done of fecal specimens and pelt swabs using Mouse PRIA (PCR Rodent Infectious Agent) Panels

4.3.22.2 Alternatively, once a year or more often as decided by the AV, blood is used for serology, and abnormal tissue (5mm slices, unless otherwise specified by veterinarian) are collected in formalin for histology, examination of the pelage and cecum/large intestines for pinworms.

4.3.22.3 Observation sheet: strain, source (vendor), age on entry, date of entry into facility, date begun to be a sentinel, protocol number, indication of what study animals exposed to, study assigned as a sentinel, and note specific rack if several racks are in the room.

4.3.22.4 Equipment/Supplies: Blood Collection Supplies: clippers, 3cc syringes with 21 (rats) and 1cc syringes with 22 (mouse) gauge needles, red top or serum separator tubes, pen marker, paper, alcohol and/or chlorhexidine swabs, small empty cage.

4.3.22.5 Serology Supplies: small container with sterile physiologic saline, pipette, yellow tip pipettes, microtubes, mailer box, ice pack, centrifuge, diagnostic lab form.

4.3.22.6 Blood Collection: blood can be collected via facial vein bleeds (mouse) if survival of the animal is desired, heart puncture or the peripheral blood vessel.

4.3.22.7 Sample preparation and shipping: Blood is centrifuged at 12,500 rpm speed for 15 minutes. Using a transfer pipette, withdraw serum from spun blood (avoid getting blood and if necessary, re-centrifuge). Also blood collection cards that uses only few drops of blood can be used.

4.3.22.8 Charles River requires 150 micro liters of serum diluted in 600 micro liters of phosphate buffered saline, that need to be stored at -20 C for one day and shipped to the laboratory, using dry ice packs.

4.3.22.9 Serology testing: Charles River – *"Assessment Plus Panel"*:

4.3.22.9.1 Rat: RPV, H-1, KRV, RMV, Sendai, SDAV, RTV, PCAR, Reo-3, LCMV, MAV, Rota-B, Hantaan, ECUL, CARB and M. pulmonis.

4.3.22.9.2 Mouse: MPV, MVM, MHV, MNV, TMEV, EDIM, Send, PVM, Reo-3, M. pulmonis, LCMV, MAV, Ectromelia,K, Polyoma, MCMV, E. cuniculi, HANT, PHV, MTLV, ECUN, and CARB

4.3.22.10 Shipping: Serum samples are to be shipped frozen using an overnight courier to Charles River.

4.3.22.11 Gross necropsy and Pinworm Evaluation:

4.3.22.11.1 Equipment: disposable scalpel blade with #10 blade, 2 hemostats, scissors, 3 forceps, dry gauze, water/alcohol/ or chlorhexidine soaked swabs, Petri dishes, 20 ml syringe with 18 needle, physiologic saline, formalin in small sterile urine collection containers, dissecting scope, sterile swabs for bacterial collection, scotch tape and slides, incubator.

4.3.22.11.2 Live animals: the sticky side of scotch tape is placed against the anal area, pressing hard, and then placed on a clean slide with sticky side down. Examine the pelage for any signs of ectoparasites and pinworms.

4.3.22.11.3 Euthanized sentinels: after the carcass has cooled, the pelage is examined for any signs of ectoparasites (section of pelt can be collected in a petri dish for exam). Wet the abdomen with water if just for pinworm collection, but alcohol or chlorhexidine if complete necropsy will be performed that may require sterile collection of tissue.

4.3.22.11.4 Incise the abdomen examining the tissue, collect any abnormal tissue, and place into a 10% formalin filled jar. Using sterile swabs, bacterial samples may be collected (pharyngeal and cecal swab). Remove the cecum and about half the large intestines, placing them into a Petri dish with ~10ml of physiologic saline. This is to be incubated for ~15 minutes at 37-40ºC. Before incubation or after, the tissue is opened and excess feces can be removed and examined using the dissecting scope looking for pinworms. Record findings.

4.3.22.12 After the result of the testing is received, the Attending Veterinarian will decide the appropriate course of action.

4.4 Quarantined Animals Health Surveillance

4.4.1 During quarantine, animals are observed daily by trained personnel for signs of sickness or injury.

4.4.2 Entry procedures for the quarantine room will be placed at the entrance and will be observed by all personnel accessing the room.

4.4.3 All rodents arriving into the quarantine room should be given a physical examination by a laboratory animal technician either during the animal receipt or during the acclimation period.

4.4.4 General assessment criteria are: behavior, appearance, presence and aspect of discharges, presence of lesions or physical injury, etc.

4.4.5 If any abnormal clinical observations are identified, the Attending Veterinarian will be notified, and an appropriate course of action will be determined.

4.4.6 Instead of using sentinel animals in a typical 6-week sentinel-based quarantine program, a Mouse PRIA (PCR Rodent Infectious Agent) Panels will be used to enable a direct screen of incoming animals just days after arrival. Required samples will be collected from live animals and submitted to the laboratory.

4.4.7 After the result of the testing is received, the Attending Veterinarian will decide the appropriate course of action.

4.5 Animal Health Checks

4.5.1 During, but not limited to, A.M and/or P.M checks, every animal caretaker should observe each individual animal for signs of illness, injury, abnormal behavior, or death.

4.5.2 Upon finding an animal with any abnormality, inform the attending veterinarian.

4.5.3 Observation of any life-threatening problem should be immediately reported to ensure appropriate and timely delivery of veterinary medical attention.

4.5.4 The attending veterinarian will then inform the researcher and take action accordingly.

5. References

5.1 Charles River - Animal Health Surveillance (http://www.criver.com/en-US/ProdServ/ByType/ResAnimalDiag/Pages/animal_health_surveillance_mouse.aspx)

6. Version History

6.1 Version 2 (January 2013): 4.3.4 was modified to include the mouse strain CD1; 6.3.26 paragraph has been added to allow the use of Mouse PRIA Panels.

6.2 Rev 03 (June 2016) clarification of the type and frequency of health screening.