A. Purpose

The purpose of the rodent health monitoring program is to evaluate the health status of incoming and existing specific pathogen free (SPF) rodent colonies at Washington State University, to rapidly detect an infectious disease outbreak within these colonies, and to prevent disease transmission between colonies due to animal transfers within the WSU campus and from external sources. For information regarding incoming rodent shipments, please refer to section #8 below for guidelines for entry and quarantine of rodents from non-commercial sources.

The rodent health surveillance program is administered by the Office of the Campus Veterinarian (OCV) with coordination and cooperation from principal investigators and vivarium management.

B. Guidelines

1. Recommended surveillance

Rodent health surveillance is necessary for all long-term rodent colonies with rats, mice, hamsters, guinea pigs, gerbils and other rodents. Short-term rodent colonies will be evaluated by the Office of the Campus Veterinarian to determine if they are exempt from surveillance depending on their health status, housing, use, and origin. The following are examples of criteria that may result in a colony being determined as exempt from surveillance.

a. The colony will only be in existence for 4 weeks or less.
b. All of the animals within a short-term colony will be eliminated and the room completely emptied and sanitized before any new animals are placed.

c. Animals from the untested colony will not share laboratory space or equipment with animals from long-term colonies.

d. The colony animals will be treated with hazardous agents or substances that may contaminate the sentinel animals and pose a health risk to animal care personnel.

2. Direct Sampling of Colony Animals for Rodent Pathogens

Colony animals may be tested directly by various methods including PCR on tissue, feces or pelt swabs, serology, fecal examination and anal tape testing for internal parasites, necropsy with histopathology, and pelage examination for external parasites. Direct testing is conducted on newly imported quarantine animals or during an outbreak investigation, but is cost prohibitive to do direct sampling in large rodent colonies. Thus, environmental or sentinel rodent testing would be used (see below).

3. Environmental Sampling of Rodent Pathogens

PCR-based diagnostic tests are available for numerous rodent viruses and bacteria as well as internal and external parasites. Pathogens can be detected by swabbing room and equipment surfaces that accumulate dust within an animal room. Examples of these surfaces are ventilated rack plenums, cage changing stations and exhaust vents. PRC-based environmental sampling may be used in replacement of or in addition to direct colony animal or sentinel rodent testing.

4. Use of Sentinel Animals for Detection of Rodent Pathogens:

a. Origin
Sentinel rodents should originate from approved commercial SPF rodent vendors and be free of known pathogens. The Office of the Campus Veterinarian must review and approve the use of sentinel rodents from other sources such as in-house breeding colonies. Sentinel animals are typically not tested prior to the exposure period.
b. **Species and Sex**

The sentinel rodents should be the same species as the colony to be evaluated (i.e. rat colonies should have rat sentinels). With exotic rodents for which no direct serological tests are available, the closest-related rodent will be used as a sentinel (i.e. deer mouse colonies have laboratory mouse sentinels). Female mice and rats and mail hamsters are preferred because of their reduced incidence of aggression and injury. If male rodents are necessary for a specific study, then male sentinel rodents may be used. Male sentinel mice are prone to fighting and may be set up in separate cages to prevent injury. Please refer to the enrichment policy for singly housed mice.

c. **Strain**

For mice and rats, outbred females such as CD-1 and Swiss Webster (mice) or Sprague-Dawley (rats) are recommended because of their robust immune response. Other mouse and rat strains may be used if they are determined acceptable by the Office of the Campus Veterinarian. Some inbred rodent strains have immune deficiencies and varied disease resistance, thus are ineffective as sentinels.

d. **Age**

Mice and rat sentinels are ordered and placed between 3 to 5 weeks of age. After placement, the sentinels are exposed for a minimum of eight weeks.

e. **Number of Sentinels and Identification**

The number and placement of sentinel rodents in the animal facility varies depending on the type of housing, research use, source of animals, and the risk of infectious transmission. OCV, in collaboration with principal investigators and vivarium management, will determine the number and placement of the sentinel rodents for each testing period. In general, there should be a minimum of one cage with 2 co-housed sentinel rodents per 50-90 mouse cages or 30-60 rat cages. In multiple investigator rooms housing rodent breeding colonies in closed microisolator caging on ventilated rack systems, each investigator colony should have its own sentinel cage.

Two sentinels are used per cage so that (1) there is a spare if one animal dies during the monitoring period, (2) one animal can be tested and serum from the second animal saved in the event of an equivocal or positive result from the first animal, and
(3) to provide social housing for social species in accordance with the IACUC Environmental Enrichment Policy.

Sentinel cages must be labeled with the strain, source, date of sentinel placement and initial exposure, exposure type (see #5: 100% bedding, rotation, or direct), date of birth, sex and ASAF number. The cage card indicates that use of the sentinel rodents is not allowed for any research purpose. Sentinels that are confirmed uninfected through testing, or those that were not utilized, may be transferred to another ASAF for other use.

f. Caging, Housing, and Handling of Sentinel Rodents
Dirty bedding sentinel rodents will be housed in sold bottom caging to facilitate exposure to dirty bedding and pathogens, with the cage size meeting minimum Guide requirements for the species. If the colony cages are open-top or suspended wire cages then the sentinel cage should be open-top as well. If the colony cages are closed with a microisolator filter top lid (ventilated or static) then the sentinel cage should also be closed. All sentinels should be provided caging enrichment as described in the WSU IACUC Environmental Enrichment Policy #30.

Handling of sentinel animals (e.g. cage changing, examination, etc.) should be performed after care has been provided for all other animals in the room. Once a group of sentinel rodents has been placed in a room on a particular rack, they must remain with that same group of animals. Sentinels shall not be moved from room to room and/or rack-to-rack between different populations/sources of rodents.

g. Exposure
Dirty Bedding Sentinel Sampling: A common surveillance method is indirect surveillance using dirty bedding. Sentinel rodents are to be housed on a composite sample consisting of 100% dirty bedding from colony cages for a minimum period of 8 weeks from the first exposure. There should be no clean bedding in a sentinel cage – only soiled bedding, urine, and feces from the colony cages. For large colonies (50-90 cages), small samples of dirty bedding should be transferred to the sentinel cage i.e. 1-3 tablespoons per cage. For smaller colonies, a larger volume of dirty bedding should be transferred to insure adequate bedding material in the sentinel cage. EVERY TIME the colony cages are changed in the room, samples of dirty bedding should be removed from EVERY changed cage and placed in the sentinel rodent cages. Longer exposures of 10-12 weeks are encouraged and
improve the likelihood of detecting an infection. If a large number of cages need to be sampled into a single sentinel cage, the use of a rotation system can be discussed with the OCV veterinary staff. When a rotation system is established, it is critical that all the cages within the colony are sampled within 4 weeks to allow seroconversion to occur in the sentinel animals before they are tested. If sentinel animals are maintained in 100% dirty bedding as described, the sentinel rodent bedding should never be clean. This ensures that the sentinel rodents are exposed to all potential pathogens in the room.

*Direct Contact and Direct Colony Sampling:* Some viruses and agents transmit poorly through contaminated bedding (Helicobacter, mouse norovirus and some external parasites). If needed, contact sentinel animals which share housing with the colony animals, or colony animals themselves, may be tested.

*Replacement of Sentinels:* When sentinel rodents are removed by the OCV staff for routine rodent health monitoring, new replacement sentinel rodents should be ordered and placed within 1-2 weeks to maximize the exposure period for the next testing period.

5. **Testing Frequency**

In most WSU SPF vivaria OCV will test for rodent pathogens every trimester using one or more of the following testing strategies: PCR-based environmental sampling, direct colony animal sampling or sentinel rodent testing. If using rodent sentinels, all sentinel animals should have a minimum of 8 weeks of exposure and preferably 10-12 weeks of exposure before testing to allow time for seroconversion and to develop a detectable parasite infestation. The testing of SPF mice, rats and related rodent species includes serology and/or PCR or viruses, bacteria, and parasitology. Additional PCR testing for various disease agents is conducted as needed using environmental sampling, direct colony animal sampling and/or sentinel rodent sampling. OCV will process the majority of sentinels a minimum of 3 times per year (roughly every 4 months). Depending on risk, some colonies will be tested more or less frequently. A few isolated rodent colonies will be tested semiannually or annually, and other more vulnerable colonies may be tested quarterly or more often.

During an outbreak or in higher risk situations we may need to collect blood, feces or fur swabs from live colony or sentinel animals multiple times during an exposure period.
Testing results are sent directly to vivarium managers and a summary of the Rodent Health Monitoring results for all vivaria will be sent to all rodent users and vivarium managers.

6. In the Event of Positive Results
OCV will retest any unexpected positive or equivocal test results, when possible, whether from environmental sampling, direct colony animal sampling or sentinel rodent testing. Once an outbreak is confirmed, OCV will institute quarantine on the affected colony and establish steps to identify the source and extent of the outbreak and to eliminate/control the infectious agent. It may be necessary to establish a “zone of suspicion” around the affected area. This may extend into laboratories in other parts of the building or campus. Suspect rooms will be under increased scrutiny and both sentinel and colony animals may have to be sampled repeatedly. It may be necessary to rederive or cull and restock the colony.

7. Entry and Quarantine of Rodents from Non-Commercial Sources
   a. Non-Commercial Sources
      Non-commercial sources include colleges, universities, research facilities, medical institutes, pet stores, Jackson Laboratories research division, trapped wild rodents and any other source that is not an OCV approved SPF vendor. Approved vendors include: Charles River Laboratories, Taconic, Envigo, Jackson Laboratories commercial division, and Simonsen. Contact OCV for a list of other acceptable vendors.
   b. Preparation
      Contact OCV at ocv.animal.shipping@wsu.edu when considering obtaining rodents from non-commercial sources. OCV works directly with the investigator, vivarium manager and non-commercial source for obtaining necessary information pertaining to receiving requested animals.
   c. Approval
      OCV reviews and evaluates the health status of the animals and determines the appropriate quarantine and treatment procedures and facility order of entry before approving the animal shipment. We strongly discourage investigators from acquiring animals with known contaminants. Incoming animals that do contain known contaminants or are coming from a facility with a history of known contaminants may require rederivation. In addition, investigators must have prior
approval from the WSU IACUC before any animal acquisition and use. A Materials Transfer Agreement (MTA) arranged through the WSU Office of Commercialization is required if the transfer is an intellectual property exchange.

d. **Housing of Quarantined Animals**
   House quarantined rodents separately from colony animals to prevent cross-contamination. Quarantined animals are considered suspect for infectious diseases and are not moved into colony rooms, procedure rooms or laboratories. Personnel must follow recommended containment procedures including PPE and order of entry.

e. **Testing**
   Testing will be done primarily via direct sampling on the imported animals for PCR testing, but sentinel animals may be used if necessary. For sentinel use, place two sentinel rodents with the imported animal shipment upon arrival for a minimum of 6 weeks for dirty-bedding or contact sentinel surveillance as described in section #5. OCV may require longer quarantine periods for treatment and optimal sentinel exposure. At the completion of the exposure period, one of the two sentinels will be tested and the remaining sentinel(s) will stay with the imported animals until the testing results are complete in the event that retesting is required.

f. **Results**
   Once the testing results are complete, OCV will indicate whether the animals may be released from quarantine and placed with the existing WSU colony. In the event of positive testing results, it may be necessary to retest, treat, rederive or reorder the shipment.

g. **Cost**
   Principal Investigators (PI) acquiring rodents from non-commercial sources with the intent to house them in WSU facilities are responsible for the costs of diagnostic testing incurred for the health evaluation as well as the quarantine *per diem* charge and treatment costs. The PI is also responsible for the cost of rederivation of the colony if that is deemed necessary.

8. **Contact Information**
   If you require any help or clarification, please call: