WASHINGTON STATE UNIVERSITY

Washington State University *Institutional Animal Care and Use Committee*	
Policy #14	**"WSU Animal Colony Health Surveillance and Quarantine Program"**
Approval Date: 5/29/2024 (Replacing Version 3/21/2021)	

A. Purpose

The purpose of the WSU animal colony health surveillance and quarantine program is to evaluate the health status of incoming and existing specific pathogen free (SPF) 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 WSU from external sources. This policy details the program and describes the requirements for participation. This policy primarily applies to rodents and zebrafish, but may be extended to other species including swine, rabbits and aquatic species as needed.

B. Policy

The WSU animal colony health surveillance and quarantine program is under the oversight of the Attending Veterinarian as defined by the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act.

The program is administered by the Office of the Campus Veterinarian (OCV) with coordination and cooperation from principal investigators and facility management. All WSU animal housing facilities are expected to participate as necessary. OCV maintains an IACUC-approved ASAF for sentinel animals

For information regarding incoming animal shipments, please refer to section #8 below for guidelines for entry and quarantine of animals from non-commercial sources.

C. Procedure

1. Surveillance Program: Zebrafish

Health surveillance is necessary for SPF colonies of zebrafish. In flow-through systems, unintentional breeding produces offspring that are occasionally found as adults in the sump tanks. These sump fish are used as the sentinel fish as they are exposed to all the water in the system. These fish will be manually caught from the sump system and will be euthanized and submitted for diagnostic testing one to four times/year depending on risk. If no sump fish are present, individual colony animals will be selected. When applicable, environmental and surface samples may be collected for PCR and culture.

2. Surveillance Program: Rodents

Rodent health surveillance is necessary for all long-term rodent colonies with SPF 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 experimentally treated with infectious agents or hazardous substances that may contaminate the sentinel animals or pose a health risk to animal care personnel.

1. Environmental Sampling of Pathogens

PCR-based diagnostic tests are available for numerous viruses and bacteria as well as internal and external parasites. Pathogens can be detected using pathogen binding filters or by swabbing room and equipment surfaces that accumulate dust. For aquatic species, environmental sampling of water or tank detritus may be used. PCR-based environmental sampling is the primary mechanism for rodent health surveillance at

WSU and may be used in conjunction with direct colony animal or sentinel testing depending on the pathogen of interest and housing situation.

2. Direct Sampling of Colony Animals for 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 or wet mount for external parasites. Direct testing is conducted on newly imported quarantine animals or during an outbreak investigation, but it is cost-prohibitive to do direct sampling in large colonies. Thus, environmental testing is more commonly used (see below).

3. Use of Sentinel Animals for Detection of Pathogens:

a. With the development of PCR-based environmental diagnostic methods, sentinel testing is rarely used but may be necessary for specific pathogens and housing situations. The Office of the Campus Veterinarian will determine when sentinel rodent use is needed.

b. Animal 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 may be tested prior to the exposure period depending on source and health status.

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 male hamsters are preferred because of their size and reduced incidence of aggression and injury. Male sentinel mice are prone to fighting and may be set up in separate cages to prevent injury. Please refer to the IACUC Environmental Enrichment Policy #30 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 strains may be used if they are determined acceptable by the Office of the Campus Veterinarian. Some inbred and genetically altered rodent strains have varied disease resistance, thus are ineffective as serology sentinels but may be useful for PCR testing.

d. Age

Mice and rat sentinels are optimally placed between 3 to 5 weeks of age and 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 multi-investigator rooms housing rodent breeding colonies in closed micro-isolator 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 samples 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 #30.

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. 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. 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. 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: 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. 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 needs 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. The majority of the sentinels will be processed 3 times a year (roughly every 4 months).

Direct Contact and Direct Colony Sampling: Some viruses and agents transmit poorly through contaminated bedding (*Helicobacter* spp., 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 monitoring, new replacement sentinel rodents should be ordered and placed within 1-2 weeks to maximize the exposure period for the next testing period.

6. **Testing Results**

   Testing results are sent directly to vivarium managers and a summary of the Health Monitoring results for all vivaria will be sent to all users and vivarium managers.

7. **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 testing. Once an outbreak is confirmed, OCV will institute quarantine on the affected colony, notify all relevant personnel 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 relocate the colony to an alternate housing area or to rederive or cull and restock the colony.

   During an outbreak or in higher risk situations, OCV may need to collect blood, feces or swabs from live colony or sentinel animals multiple times during an exposure period.

8. **Entry and Quarantine of Rodents & Zebrafish 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 rodent vendors include Charles River Laboratories, Taconic, Envigo/Inotiv, and Jackson Laboratories commercial division. Contact OCV for a list of other acceptable rodent and for zebrafish vendors (ZIRC).

   b. Preparation

      Contact OCV at ocv.spokane.vet@wsu.edu (Spokane only) or ocv.animal.shipping@wsu.edu (Pullman and other campuses) when considering obtaining animals, cryopreserved embryos or sperm from non-commercial sources. OCV works directly with the investigator, vivarium manager and non-commercial source to obtain necessary information pertaining to receiving the requested animals. For rodents entering IVS facilities see the IVS non-commercial source guide addendum.

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. When available, OCV will also review health status for sources of cryopreserved embryos and sperm. We strongly discourage investigators from acquiring live animals with known pathogens. Incoming animals that do contain known pathogens or are coming from a facility with a history of known pathogens may require rederivation. In addition, investigators must have prior approval from the WSU IACUC https://iacuc.wsu.edu/forms/ before any animal acquisition and use. A Materials Transfer Agreement (MTA) arranged through the WSU Office of Research Support and Operations (ORSO) (https://commercialization.wsu.edu/mtas/) is required if the transfer is an intellectual property exchange.

d. Housing of Quarantined Animals

House quarantined animals 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. Imported zebrafish should not be directly placed on to colony flow-through systems. Specific pathogen-free offspring can be derived by disinfecting embryos from imported parent stock. Personnel must follow recommended containment procedures including personal protective equipment (PPE) and order of entry.

e. Testing

Testing will be done primarily via direct sampling on the imported animals for PCR testing, but environmental sampling or sentinel animals may be used if necessary. Recipient female rodents used for embryo transfer rederivations from suspect imported embryos or semen will be screened via serology and/or PCR as needed. For rodent 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. Zebrafish derived from bleached embryos may require testing as determined by source and incoming health reports.

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 or identify an alternate source.

g. Cost

Principal Investigators (PI) acquiring animals 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* charges and treatment costs. The PI is also responsible for the cost of rederivation of the colony if that is deemed necessary.

9. Contact Information

If you require any help or clarification, please contact:

- Office of the Campus Veterinarian (all campuses): 509-335-6246 or ocv.animal.shipping@wsu.edu
- OCV Program of Laboratory Animal Resources (WSU Spokane): 509-358-7825 or ocv.spokane.vet@wsu.edu
- Animal Welfare Program (IACUC): 509-335-5353 or iacuc@wsu.edu