



Title: Rodent Health Surveillance Program

SOP Number: 057

Purpose: The objective of this Rodent Surveillance Health Program is to provide the highest quality of care for rodents housed in TTU Animal Care Services (ACS) Facilities. These programs are necessary to prevent the introduction and transmission of pathogenic organisms, isolate & contain potential sources of infection pending confirmation of test results and contain, treat and eradicate outbreaks.

TEXAS TECH UNIVERSITY IACUC POLICY POSITION

Rodents are subject to several viral, bacterial and parasitic infestations that can impact their usefulness in research. The IACUC has established a Rodent Health Surveillance Policy (Policy 06) to safeguard the health and pathogen-free status of all TTU-maintained research animals.

- A. All newly received animals will be subject to a basic health evaluation.
- B. Animals that arrive from other than well-known, reliable commercial vendors will be subject to quarantine.
- C. Sentinel rodents will be used to ensure that all ACS animal rooms remain pathogen free.
- D. All rodents obtained or transferred from other than approved commercial vendors must undergo a minimum 6–8-week quarantine period during which they will be screened and treated for a variety of common pathogens.
 1. This mandatory isolation period is required regardless of any animal health screening information that may be provided by the transferring institution.
- E. Use of/contact with quarantined animals is strictly limited.
 1. Research personnel may only access the quarantine housing rooms if prior arrangements have been made with (ACS) veterinarians or staff.

BACKGROUND AND RATIONALE

The rodent research program at TTU is incredibly varied in terms of academic disciplines, type of scientific investigation, geographic distribution of facilities, and physical structure of facilities. The research personnel at TTU have a large diversity of background and experience, as well as very large numbers of academic and scientific staff (animal users).

An important aspect of ensuring quality results from research performed in rodents is providing assurance that experimental results are free from interference by infectious disease of rodents. By establishing a sentinel program, continued assurance can be guaranteed for all of the rodents housed in ACS facilities. Although quarantine programs are costly and may appear cumbersome to research staff in terms of time and convenience, the positive benefits in terms of disease prevention and therefore successful research overcome these perceived drawbacks. For all of these reasons well-defined and strictly maintained preventative health programs for rodents is essential.

It is widely accepted that the greatest risk to established colonies is in the introduction of animals from outside sources. With the increased distribution of animals between academic

institutions, owing to the development of transgenic lines, the risk becomes intensified because the health status of exporting colonies is not equivalent; certain colonies exclude pathogens that others do not, and colony health monitoring and maintenance programs may vary widely between institutions.

CURRENT APPROVED COMMERCIAL VENDORS

- A. Charles River
- B. Envigo
- C. Taconic
- D. Jackson Labs

SENTINEL ANIMAL PROGRAM

A. Colony Animals

1. May be tested directly by various methods including
 - a. PCR on tissue, feces, or pelt swabs,
 - b. Serology
 - c. Fecal examination and anal tape testing for internal parasites
 - d. Necropsy with histopathology
 - e. Pelage examination for external parasites.

B. Environmental Sampling

1. Pathogens can be detected by
 - a. Swabbing room and equipment surfaces that accumulate dust within an animal room.
 - b. PCR-based environmental sampling may be used in replacement of or in addition to direct colony animal or sentinel rodent testing.

C. Sentinel Numbers, Housing and Exposure

1. Two CD-1 female mice 3-5 weeks of age (Charles River) or two CD female rats 4-6 weeks of age (Charles River) are placed per sentinel cage.
2. One cage of sentinel mice/rats per rack are placed on the bottom row of the racks or shelves being used.
 - a. In general, one sentinel cage per 50-80 mouse cages or 30-50 rat cages.
3. Sentinel mice/rats are kept for 6-8 weeks before testing.
4. Place clean bedding in the box half the amount that's normally filled. The other half is filled with dirty bedding from other cages on the rack.
5. Soiled bedding from each cage on the rack is collected into the sentinel cage and mixed in with the bedding. The composite sample should always include feces and urine (the sentinel cage should never look clean). Feed and water are collected from cages on the rack during change outs and used to fill the hoppers and water bottles of the sentinel cages as well. 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.

D. Sentinel Testing

1. ACS will test for rodent pathogens quarterly using one or more of the following testing strategies
 - a. PCR-based environmental sampling,
 - b. Direct colony animal sampling, or
 - c. Sentinel rodent testing.
2. Parasitology-in house fecal floatation; Annual pinworm and fur mite PCR
3. A perianal cellophane tape specimen is examined for pinworm ova in house.
4. Fecal Exam – specimens from all Sentinels tested from each room are pooled and examined for endoparasites in house. Annual PCR.
5. A cellophane tape specimen from the perianal area and a fur pluck from the nape of neck are examined for ectoparasites. Annual pelt swab PCR.

E. Serology

1. Blood is collected from the submandibular vein or tail vein and placed on an Opti-Spot card and is then sent for a serology panel to IDEXX Laboratories on a quarterly basis.
2. A whole animal per room may be sent to Charles River Laboratories or IDEXX.
3. The following tests are routinely performed as part of the ACS panel for health evaluation for mice:

Sendai	MHV	M. pul	PVM
MVM	TMEV	REO3	LCMV
ECTRO	EDIM	MPV	MNV
MAD1	MAD2		

4. The following rat panel of tests are routinely performed:

Sendai	PVM	SDA/RCV	KRV
H-1	REO-3	M. pul	LCMV
CARB	RPV	RMV	RTV

5. Histopathology – specimens are collected on grossly “abnormal” tissues only. Tissues are sent to a histology laboratory for evaluation. Reports are sent back to the Veterinarian.
6. At the discretion of the veterinarians, necropsies ± histopathology may be performed on unexpected or suspicious rodent deaths.

STEPS TO FOLLOW FOR INCOMING RODENT QUARANTINE ANIMALS

A. Primary Investigator (PI)

1. Before beginning the process, contact the sending PI for the shipping contact information for the animal order.
2. Submit a new Animal Request Form
3. Please fill in the vendor area as “Other”.
4. When filling in the contact information please use the **shipping contact** from the other institute.
5. When listing the rodents to be ordered you will have to put each strain and gender on their own line in order to count for them all.

6. If there is any paperwork from the institution sending the rodents, please forward it to the facilities manager. It will be completed and sent back to the requestor as part of the ordering process.

B. Ordering Process (Animal transport personnel)

1. ACS will request a health report and a description of the colony and husbandry practices at the vivarium room of origin, from the sending institution.
2. Must have negative sentinel results from the last 90 days from the same animal housing room as well as the latest facility results, health records must be negative the following pathogens:
 - a. **Mice:** Ectromelia, EDIM, LCMV, *M. pulmonis*, MAD1, MAD2, MHV, MNV, MPV, MVM, PVM, REO3, TMEV, Sendai, *Helicobacter* sp. + Pinworms and mites
 - b. **Rats:** PVM, CARB, *M. pulmonis*, REO3, Sendai, LCMV, RCV, RPV, RMV, Kilham Rat Virus, H-1 Virus, RTV, *Helicobacter* sp. + Pinworms and mites
3. Once information is received, the Attending Veterinarian (AV), or their designee will review and approve or disapprove the shipment.
4. Correspondence may be necessary to confirm shipment health status and further testing may be required before the animals are allowed into TTU animal facilities.
5. Any additional testing requirements will be at the cost of the investigator and must be approved prior to coordinating with the sending institution.
6. Once health status is approved, the order will be approved, and the animals will be shipped.
7. The Investigator and sending institution will be emailed concerning the approval and the animal order going forward.
8. Shipping arrangements will be made by the facilities manager who will update the PI as shipment is arranged.

NOTE: Animal orders will not be submitted without veterinary approval based upon source health report review.

ANIMAL RECEIVING

- A. Upon arrival, inspect the shipping crates to be sure that all are in good condition.
- B. Take the crates to the quarantine room and take pictures of the crates to document the condition. The packing slips from the crates go to the ACS office.
- C. Decontaminate the outside of the shipping container with disinfectant (PeroxiGard or other approved disinfectant).
- D. Remove rodents from the shipping container under the Biosafety Cabinet in the quarantine room.
- E. Place rodents into prepared cages and cage cards under the Biosafety Cabinet. Carefully match the information from the labels on the crates to the cage cards.
- F. Any discrepancy between the information on the crate and the cage card should be reported to the facilities manager.

- G. Obvious differences between the information and the animal should be reported as well. For example, if black mice are expected and the mice are white, this would be an obvious discrepancy and should be reported.
- H. An accurate count of animals, including a count of dead animals and gel packs or water kits is reported on the daily observation sheet which is located in the quarantine room.
- I. Animals are dead on arrival:
 - 1. Leave dead animals in the crate and notify an ACS veterinarian asap.
 - 2. The technician will photograph the dead animal(s) along with the crate label.
 - 3. The technician will discuss with the veterinarian whether or not a necropsy of the animals will be carried out.

QUARANTINE FACILITY/ANIMAL HUSBANDRY

- A. The quarantine room for all incoming rodents obtained from “outside” or non-approved vendor sources will be physically isolated from standard rodent housing rooms within the destination vivarium on the main TTU Campus.
 - 1. Separate per diem rates will apply to all quarantine animals, in addition to testing requirements (see below), and fees.
- B. A dedicated technician will be assigned to the room and daily animal care in this room is performed as the final task of the day.
- C. Rodents will be observed at least once daily
- D. Animals with any clinical signs of disease or illness will be reported to the animal health technician or veterinarians.
- E. If death occurs, place the carcass in a labeled biohazard bag in the refrigerator and contact an ACS veterinarian immediately for potential necropsy and sample collection.
- F. All rodents are maintained using typical BSL-2 environment husbandry techniques.
 - 1. Observe proper hygiene, and be aware of allergy, zoonosis and injury risk at all times (Refer to the [TTU-OH&S](#) webpage for more information).
 - 2. In addition to trash and dirty bedding, all dirty caging is autoclaved before being washed.
- G. Disposable PPE (disposable gown, cap, mask, shoe covers and gloves) are worn for each entry and discarded before exiting the room.
- H. Gloves are changed between cages.
- I. The most recently acquired or the most highly suspect animals within the room are serviced last.
- J. Weekend and holiday checks/care are performed as the final task of the day by the assigned duty technician.
- K. After entering the quarantine area, husbandry personnel are not permitted to enter any other animal housing area until the next day and must shower during the interval.
- L. Quarantine time is approximately 6-8 weeks.
- M. Upon completion of quarantine the Investigator will be notified, and the animals will be transported to their final destination.

QUARANTINE ANIMAL TESTING

- A. The quarantine room is surveyed for murine pathogens including MHV, MNV, MVM, MPV, Sendai virus, *M. pulmonis*, Reo-3, PVM, TMEV, LCMV, ECTRO, EDIM, MAD1, MAD2, *Helicobacter* sp., pinworms and mites.

- a. For rats, the quarantine room is surveyed for pathogens including Sendai, PVM, RCV, KRV, H-1, REO3, M. pul, LCMV, CARB, RPV, RMV, RTV, *Helicobacter* sp., pinworms and mites.
 - 1. Upon arrival into quarantine, a fur pluck test and tape test will be conducted on one animal per cage to screen for mites and pinworms and a fecal pellet will be evaluated for *Helicobacter* sp. by fecal PCR or mice will be swabbed and feces collected for fur mite and pinworm PCR.
- B. Sentinel animals at the investigators cost are placed in the room and exposed to potential pathogens by exposing them to soiled bedding twice per week from other rodents occupying the room.
- C. Rodents are placed on a Fenbendazole diet (unless PCR tested upon arrival for pinworms and fur mites) and tested for murine pathogens as indicated by the previous health report and for the facility, they will eventually be housed.
- D. Sentinel animal/sera for viral serology and parasite panels will be submitted to a reference laboratory for analysis at 6-8 weeks. Rodents will not be released to the PI until tests results are completed and an ACS veterinarian clears the animals to enter the colony.
- E. The cost of the quarantine testing will be charged to the investigator directly.

PROPHYLACTIC PARASITE TREATMENT DURING QUARANTINE

- A. Pinworms: Fenbendazole feed treatment as follows:
 - 1. Treat all animals with 150 ppm containing diet
 - 2. Maintain in the feeder for the entire quarantine period
 - 3. Sentinels will not be treated
- B. Fur Mites:
 - 1. Mite arrest (7% pyrethrin) applied to nesting material.
 - 2. Treat ALL ANIMALS at the same time.
 - 3. Sentinels will not be treated
- C. An alternative is to PCR test for pinworms/fur mites prior to arrival or at arrival.

WORKING WITH YOUR ANIMALS IN QUARANTINE

- A. Except in special situations, research personnel may not enter the quarantine room during the quarantine period.
- B. Experimental treatments cannot be initiated during the quarantine program unless exceptional circumstances apply, and clearance has been obtained through the Director of ACS/AV and the IACUC.
- C. Rodents are not allowed to breed in quarantine until they have been in quarantine for three full weeks, thus are only allowed to start breeding at week four.
 - 1. Any pups born prior to week four of quarantine period are euthanized by ACS staff.
 - 2. Any exceptions where pups may be taken directly to the laboratory for euthanasia must be pre-approved by the IACUC and the Attending Veterinarian
- D. With prior veterinary approval, rodents may be moved from the quarantine facility to an investigator's lab for acute, terminal studies only.
 - 1. Animals may not be housed for more than 12 hours in an investigator's lab.

E. Animals that leave the quarantine facility will never be allowed to re-enter any ACS vivarium.