Rodent Husbandry SOP

Last Updated 12 May 2016

1. Purpose

   Procedures for the care and monitoring of rodent (except guinea pigs) health in animal rooms.

2. Responsibility
   a. Husbandry Personnel
   b. Veterinary Technicians

3. Definitions

   Concerning all procedures that begin with (REQ)

   Procedures that begin with (REQ) indicates the procedure is required to be followed by all ULAM and non-ULAM laboratory animal departments. All other procedures not beginning with (REQ) are still required to be followed by ULAM but may not be adopted by non-ULAM laboratories.

   a. Acetate: A colored, plastic card placed over animal cage cards that aid in communication between husbandry, ULAM veterinary and investigative personnel.

   b. Abnormal: Condition departing from the normal strain specific expectations; irregularity.

   c. Animal Treatment Report (ATR): Reports note an animal health condition and are completed by husbandry personnel or investigative personnel and given to veterinary personnel for animal assessment.

   d. Bright, Alert, Responsive (BAR): Used to describe a healthy rodent.

   e. Business Days: A business day is defined as any day during the week beginning Monday through Friday, including Thanksgiving Day, the Friday after Thanksgiving, and the 4 season days recognized by the University.

   f. Cage Cards: 3”x5” card affixed to the exterior of the cage. Cage cards may be white, yellow, pink, blue. Yellow cards indicate litter births, litter weaning and pup deaths. White cage cards indicate animals arrived from a vendor. Pink cage cards refer to a weaned female litter or female animals. Blue cage cards refer to a weaned male litter or male animals.

   g. Dystocia: Slow and difficult labor or delivery.

   h. Enrichment: The use of structures and resources to encourage species specific behaviors and to enhance psychological health and well-being of various species defined in this document according to the requirements of The Guide 8th edition.

   i. Food Chewer: Mice who consistently chew or shred chow; this behavior causes the bottom of the cage floor to be filled with excessive amounts of food dust and/or chunks.

   j. Healthy Pup: A young animal that is bright, alert, responsive, hydrated and otherwise does not appear sick.

   k. Micro-Isolation Technique (MI): A method of housing and handling animals to maintain them free of disease-causing pathogens.

   l. Moribund: Means any condition where death is imminent.

   m. Neonatal Pups: Rat or mouse pups without fur, eyes not open (approximately 10 days old or less).


   o. Nesting Material: Commercially available shredded paper (e.g. Crinkle nest, Enviropod or Enviropacks), nestlets, Alpha Dry or Carefresh bedding.

   p. Opaque Cages: Cages that are not transparent but receptive to changes in light, generally used to minimize animal stress levels.
q. **Specific Pathogen Free (SPF):** Animals are free of certain pathogens and are housed in cages with micro-isolator tops.
r. **Spor-Klenz RTU©:** A disinfectant containing hydrogen peroxide and peroxyacetic acid. It is bactericidal, tuberculocidal, virocidal, and fungicidal.
s. **Special Treatment or Procedures Form (SToP):** Form indicates exceptions to normal husbandry procedures, approved by the laboratory, husbandry personnel and/or UCUCA.
t. **Unhealthy Pup:** A young animal that is hunched and scruffy, quiet, lethargic or otherwise appears sick.
u. **USDA Regulated Species:** Species covered under the the Animal Welfare Act; including Cotton Rats.

4. **Procedures**

a. **Monitoring and Providing Food and Water**

   i. **(REQ)** Assessment of the availability of food must occur daily.
      1. Provide food using a rodent standard diet (see Appendix D) if food level is below the half-way point of a feeder or wire used for feeding.
      2. Feed food chewing mice ½ the amount of food normally fed during routine cage changes.
         a. Additional food does not need to be added to the wire lid/feeder if the following conditions apply:
            i. The food on the bottom of the cage is clean, dry and mold-free.
            ii. There must be enough chunks of food on the floor to equal one piece of rodent meal per mouse.
         b. Place a yellow acetate labeled “food chower” on any cage with animals that are considered food chowers or place a yellow acetate labeled “food chower” on the door of the animal room if there is more than one cage with food chowers.
      3. Place Diet Gel® 76A if animals require it (i.e. dehydrated).
         a. Remove uneaten Diet Gel® during next cage change day.
         b. Discard any unused portion of Diet Gel®.
         c. Place unopened Diet Gel® in animal room for later use.
         d. Do not place Diet Gel® in special diet cages unless approval from the laboratory is received.
   
   ii. **(REQ)** Assess the availability of water daily.
      1. Provide water bottles to all animals housed in static cages. Ensure water is available to animals.
      2. Open top water line valves on automated water and ventilated racks daily. Close the valve once the water flow has been observed.
      3. Check placement of lixit and ensure it penetrates the back of the cage.
         a. At cage changing, test each lixit for effective function.
         b. Remove and bring leaking or broken lixits to the area supervisor for repair.
      4. Place autoclaved water bottles and remove the lixit on immune-compromised rodents located on ventilated racks. Automated water must not be provided to these cages. Place a yellow acetate indicating "autoclaved/RO water needed for immunocompromised animals”.
      5. Complete an ATR and provide a water bottle to animals on automated watering racks that appear dehydrated:
         a. Place a stickered yellow acetate indicating water bottle 7 days (i.e. Date, Monday) and/or check lixit for 7 days (i.e. Date, Monday).

b. **Observing Rat and Mouse Health**

   i. **(REQ)** Observe rodents for the presence of the following conditions daily:
      1. BAR
      2. Posture and Movement
      3. Behavior
      4. Report abnormalities (e.g. lesions, tumors, masses etc.).
      5. Normal feces formation and urine output
      6. Births/deaths
      7. Overcrowded cages
   
   ii. **(REQ)** May not be able to visualize all of the above described conditions (section 4.b.i points 1 through 7) every day in the presence of a large nest. See section 4.c.i.1.a for methods of health checking the cage when you cannot fully visualize every animal individually.
   
   iii. **(REQ)** Observe, document and report any animal abnormalities on an ATR.
   
   iv. **(REQ)** Reporting animal emergencies as stated in Animal Care Identification and Communication Procedures.

b. **Perform Health Checks**

   i. **(REQ)** Perform health checks on non-cage change days.
      1. Encourage animals to move by flipping up the cage card or gently tapping the cage.
         a. Environmental enrichment may inhibit the visualization of rodents during non-cage change day health checks, however, other factors such as nest building, composition and movement within the nest can be used to determine the health of animal.
            i. **(REQ)** Monitor the cage environment for the following conditions:
               1. Conditions that suggest a healthy cage environment:
                  a. Feces and urine present
                  b. Well formed, domed nest
                  c. Cage space well organized with the nest located away from the urine site
                  d. Healthy social behavior in which animals are nesting/sleeping together
                  e. Animals are utilizing the environmental enrichment and/or nesting material
                  f. Movement with in the nest indicates active animals
               2. Conditions that indicate a negative health condition and should be monitored more closely
a. Poor quality nest that is flat or not well formed
b. Poor cage space organization in which the next is mixed in with soiled materials
c. Poor social behavior in which animals (or a single animal) is nesting separately
d. Urine stained, flat nest that may indicate aggression
e. Animals are not utilizing the environmental enrichment and/or nesting material

f. No movement in the nest even after gently tapping on cage or flipping up cage card

ii. See Appendix E for visual examples of the above described cage environment conditions

2. It is imperative that animals receive a thorough health check on cage change days (see below).
3. Use a flashlight if animals cannot be seen.

ii. (REQ) Perform health checks on cage change days by removing the animal and examining it in its entirety.
iii. Perform health checks on animal rooms with reverse light cycle during the light phase, if possible.
iv. Perform health checks on animals in opaque cages and report "Opaque health check day" on room sheet a minimum of once weekly. On all other days, visually inspect through the top of the cage and limit physical disturbances (i.e. opening cage).

1. If more than 2 adults and 1 litter are identified on opaque cage health check, follow 4.h.iii.1 or 4.h.iii.2 below.
2. Remove animals from flooded or excessively wet cages and place the animals in a dry cage with a warm heat pad underneath half of the cage, nesting material and a water bottle. The next day, remove the water bottle and place animals back in the original spot on the rack, assuring lixit is functioning appropriately.
   1. Place animals in a location where the cage can be on the heating pad and the veterinary personnel can access it easily.
   2. Generate an ATR if necessary.
   3. Remove the water bottle and place animals back in the original spot on rack the next day.

vi. Remove dead adults and pups following the procedure discussed in Documenting, Disposal and Reporting Animal Deaths.

vii. (REQ) Note observations that do not affect the health of the animal on a clear acetate. See Animal Care Identification and Communication Procedures.

d. "Do Not Disturb" Cages

i. Delay thorough health checks up to three days if the laboratory has requested a "do not disturb" period after the birth of a litter.
ii. Change cages after the do not disturb period has ended.
iii. Disturb cages if the following conditions occur: excessively wet bedding, condensation build up, animal overcrowding, adult animal death or lack of food or water.
   1. If dead pups are seen in these cases, record dead pups on cage card, however, leave dead pups in cage until after the three day period.
   2. If more than 2 adults and 1 litter are identified during this period, follow 4.h.iii.1 or 4.h.iii.2 below.

e. Managing Fighting and Fight Wounds in Mice

i. (REQ) Separate suspected fighting animals and report potential fight wounds using animal emergency procedures.
ii. (REQ) Record, aggressive mouse, house alone or something similar on cage card.
iii. (REQ) Separate the aggressor and record the location of the aggressor on an ATR.
iv. (REQ) Remove the injured animal(s) if aggressor cannot be identified.
v. (REQ) Provide a cage card with information listed in section 4.g.vi for animals that were separated.
vi. Place cage containing all mice on the rack.

f. Recording Mice and Rat Birth and Neonatal Death

i. (REQ) Record all births and deaths on the cage card.
   1. Indicate births by the date of birth only. Litter sizes are not recorded.
   2. Record the number of dead animals and the date on the same line with the corresponding birth date of the litter. This includes recording pup remnants or body parts (see Appendix F).
   3. Record missing pups with a "gone" or "no pups" designation along with the date.
   4. Record an end date of each birthed litter with either a wean date or a death date.
   5. Euthanize moribund or debilitated neonatal pups. Laboratories are not notified when neonatal pups are euthanized. Record deaths as in section 4.f.i.2 above.

i. (REQ) Healthy mouse and rat pups should be weaned at 22 days.
ii. Place a blue, "Please Separate" acetate with the date and day on any cage requiring animals to be weaned.
iii. If the lab does not wean the pups by the following business day husbandry personnel separates the animals.
iv. Wean healthy pups into same sex, socially housed cages (see Appendix B).
   1. If pups appear small, it may be necessary to place a water bottle with a longer sipper tube and/or diet gel. Place appropriate acetate.
   2. Sickly or unhealthy pups are to remain in the parent cage and generate an ATR.
      a. If leaving a single unhealthy pup with dam, where possible, leave a healthy pup of the same age and sex to facilitate social housing at weaning.
   v. In cages where a second litter is born before all pups from the first litter have reached 22 days, see 4.h.iii.3 below.
vi. Separate the pups using housing densities policy (see Appendix A). Separate by sex into appropriate cages with either a pink (females) or blue (male) cage card.

vii. Transfer all information to the new pink or blue cage card, include the following information (some of which may be on
the weanling barcode sticker):
1. New barcode sticker or include the following if a barcode sticker is not available:
   a. Principal Investigator
   b. Protocol number
   c. Account number/short code
   d. Species
   e. Strain
   f. Laboratory contact
   g. Original bar code number
   h. Number of animals in the cage
   i. Animal source
   j. Pup wean date must be noted on original and new cage cards
   k. Who weaned or separated the cage (i.e. ULAM, ULAMvt, Lab)
   l. Specific cage identification (may be placed by lab for additional identification)

   viii. Place a yellow acetate stating "check lixit for 7 days" in automatic-watering cages, include the start date and the start day (i.e. Date, Monday), on a cage with a lixit.
   ix. Place a long sipper tube on water bottles in static cages.

h. Separating Animals in Overcrowded Cages
i. **(REQ)** Place a blue acetate with the date and day on any cage requiring animals to be separated.
ii. Separation of mouse and rat cages occurs on the following business day if the cages have exceeded the maximum housing densities (see Appendix A).
iii. For separations resulting from more than from 2 adults and 1 litter of mouse pups born in a cage:
   1. If 3 adults (typically 1 male and 2 females) and 1-2 litter/s:
      a. Mark the date and day (i.e. Date, Monday) on both a blue and red acetate and place them on the cage.
      b. Write an ATR, indicating "overcrowding" or "OC" and submit to veterinary technicians.
      c. Within 24 hours a veterinary technician will examine the cage and email the laboratory, cc'ing the faculty veterinarian, that the lab has 1 business day to separate out specified mice.
      d. If the lab does not respond to the veterinary technician to confirm separation by the required time, the veterinary technician will separate out:
         i. In cages of 3 adults and 1 litter: the adult male and adult female who has not given birth.
         ii. In cages with either 2 or 3 adults and 2 litters: the older litter and associated dam.
      e. The veterinary technician will charge the laboratory for technical time required to process overcrowded ATRs, assess mice, communicate with the laboratory, and perform separations.
      f. Post-separation communication, cc'd to faculty veterinarian:
         i. Upon a laboratory's first uncorrected overcrowd, the veterinary technician should email the laboratory that the separation occurred, a technician time fee was charged, and the lab should contact their facility faculty veterinarian with any questions or concerns regarding the UM Policy on Mouse and Rat Breeding and Cage Densities.
         ii. For all additional uncorrected overcrowds, as above, the laboratory will be required to discuss the Policy with their facility faculty veterinarian.
   2. In a cage with 2 adult females and 2 litters of pups:
      a. If pups are not able to be weaned follow steps in section 4.h.iii.1.
      b. If pups are able to be weaned follow steps in section 4.h.iii.3.
   3. If a female mouse gives birth to a second litter before all pups from her first litter have reached 21 days of age:
      a. Place a blue acetate with the date and day (i.e. Date, Monday) on the cage requiring animals to be separated.
      b. If the lab does not wean the older litter out of the cage within one business day, husbandry personnel separates all pups in the older litter that are healthy, regardless of size or age.
         i. Where possible, wean smaller pups into a cage with some larger pups to assist with thermoregulation and nest building.
         ii. In addition, place a water bottle with long sipper tube, food pellets on the cage floor, diet gel, provide shredded paper nesting (i.e. CrinklePaper, Envirodry) and place a yellow acetate with "Early Wean, long sipper tube, food on floor for 7 days" with the start date and start day (i.e. Date, Monday).
         iii. Seven days later, if pups remain healthy, additional care can be discontinued and a yellow acetate stating "check lixit for 7 days" can be placed on automatic-watering cages, include the start date and the start day (i.e. Date, Monday).
      c. If any of the pups from the older litter appear unhealthy, leave them with their dam, place a red acetate on the dam's cage and submit an ATR.
         i. Veterinary Technicians will place a yellow acetate stating "Extended or delayed weaning, long sipper tube, food on floor" with the start date and day (i.e. Date, Monday) and a green acetate indicating the condition being monitored.
         ii. Where possible, leave a healthy pup of the same age and sex to facilitate social housing after weaning.
      iv. Separate animals into appropriate cages with the appropriate colored cage card. In most circumstances, adult rats will be moved to a larger cage rather than separated.
      v. Transfer all applicable information from section 4.g.vi. to the cage card.
      vi. Indicate the number of animals removed, the date and who separated the animals (i.e. ULAM, ULAMvt, Lab) on the cage card of the overcrowded cage.

   i. Changing Rat and Mouse Cages:
i. Change SPF animal cages using micro-isolator technique.
   1. Transfer some of the nesting material (if applicable, rats may not have any) from the dirty cage to the clean cage during cage change.

ii. *(REQ)* Change cages more frequently then indicated in the *(Rodent Equipment Maintenance and Sanitization SOP)* if the following conditions exist:
   1. *(REQ)* Bedding is more than ¼ wet.
   2. Excess food or bedding on the floor blocks the airflow in ventilated cages.
   3. *(REQ)* Excess food or bedding hits the sipper tube causing a static cage to become flooded.
   4. *(REQ)* Excess food or bedding in a static cage hits the wire lid restricting both space availability and movement within the cage.
   5. Condensation forms along cage walls and/or cage lid filter becomes moist with condensation.
      a. Report condensation formation to the area husbandry supervisor.
      b. The area husbandry supervisor will work with the area faculty veterinarian to troubleshoot the underlying cause and develop a management plan.

iii. Changing cages with new litters:
   1. Change cages with new litters at least 24 hours after parturition or the next change day for animals, unless the bedding in the cage is a ¼ wet or more.
      a. Transfer the pups by scooping them with some nesting material or bedding to transfer scent.
      b. Add nesting material if needed.

iv. Procedures for when SPF animals fall on the floor:
   1. Place animal in a separate cage on the rack, with a complete cage card (see section 4.g.vi above) and yellow acetate labeled "fell on floor, handle, change last", with the date. Handle this cage last until one complete round of surveillance has been achieved, then the acetate can be removed.
   2. Report as an animal emergency, if the animal is a mouse or rat pup that is too small to be weaned, place it in a cage by itself on the flow hood with a completed cage card.
   3. The Veterinary Technician contacts the laboratory to determine if the pup should be euthanized or if it should be returned to its parent cage. If returned to the parent cage, treat as in section 4.i.iv.1 above.

j. Providing Enrichment
   i. *(REQ)* Refer to *(Environmental Enrichment for Animals SOP)* for conditions in which rats and mice will receive environmental enrichment.

5. Related Documents
   a. *(Rodent Equipment Maintenance and Sanitization SOP)*
   b. *(Animal Care Identification and Communication Procedures)*
   c. *(Documenting, Disposal and Reporting Animal Deaths)*
   d. *(Environmental Enrichment for Animals SOP)*
   e. *(Small Animal Receiving)*
   f. *(Guidelines for Documenting Scientific Justification for Exceeding Cage Densities)*
   g. *(Guidelines on Mouse and Rat Breeding and Housing Management)*

6. Appendices
   a. Appendix A: *(Maximum Housing Densities for Rat and Mouse Cages)* (linked from appendices section of *Guidelines for Documenting Scientific Justification for Exceeding Cage Densities*)
   b. Appendix B: *(Handling Weanling Mice Flow Chart)*
   c. Appendix C: *(Micro-isolator Technique (MI))*
   d. Appendix D: *(Rodent Standard Chow List)*

Frequently used, commercially available.

<table>
<thead>
<tr>
<th>Lab Diet</th>
<th>Teklad Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>5L0D</td>
<td>2916</td>
</tr>
<tr>
<td>50083</td>
<td>2918</td>
</tr>
<tr>
<td>5058</td>
<td>2919</td>
</tr>
<tr>
<td>5001</td>
<td>2920X</td>
</tr>
<tr>
<td>5013</td>
<td>7012</td>
</tr>
<tr>
<td>5V5M</td>
<td></td>
</tr>
<tr>
<td>5321</td>
<td></td>
</tr>
<tr>
<td>5025</td>
<td></td>
</tr>
<tr>
<td>5326</td>
<td></td>
</tr>
</tbody>
</table>
e. Appendix E: *Tips for Health Checking with an Enviropak*

f. Appendix F: *Rodent Death Log*