Persons Responsible
All

Purpose
To establish the procedures for the entry and exit of personnel and materials to and from rooms, areas and facilities known to be contaminated with non-experimental animal pathogens.

Safety Considerations
NOTE: THE PI IS RESPONSIBLE FOR THE SAFETY AND TRAINING OF THEIR LABORATORY PERSONNEL.

Procedure
Serological testing of sentinel animals in mouse and rat colonies is performed periodically. Additional precautions are instituted when an underlying infection by detrimental animal pathogen(s) is detected in order to limit the potential for cross-contamination of other, non-infected, areas.

1. Establish room entry sequences that prioritize the performance of animal husbandry duties in non-contaminated rooms, areas and facilities prior to the performance of duties in contaminated ones.

2. Post signs at the entrance doorway of affected animal rooms, areas and facilities identifying the pathogen detected and listing precautions required for entry and exit, including, but not necessarily limited to, additional Personal Protective Equipment (PPE).

2.1 Don required PPE on entry.
2.2 Perform animal husbandry duties on non-contaminated racks or sections of racks first and on contaminated sections last, if the infection is confined to a defined section of the room, area or facility.
2.3 Change disposable gloves between each side or section of a rack and between groups of cages belonging to different investigators.
2.4 Prepare and decontaminate all equipment, supplies and materials to be removed from the room.
   - Place all autoclavable items, including soiled caging, used water bottles and trash into autoclave bags and seal.
   - Spray all sealed bags and all non-autoclavable items with a pathogen specific disinfectant.
   - Allow ten minutes contact time for maximum disinfectant effectiveness.
   - Remove sprayed items from the animal room without stepping across the threshold.

2.5 Remove PPE and store or discard, as appropriate.
2.6 Exit the animal room.
3. Cover all bagged materials with a sheet and transport to the appropriate dirty equipment storage location or to the Dirty Side of the Cage Wash facility for later transport, autoclaving and processing.

4. Take a full body shower, including hair, prior to entry into any other non-contaminated animal room, area or facility on the same day.

Author: Steven K. Cary BS, LATg–Animal Care Specialist–LAR
Reviewed by: Denise Ostmeyer BS, CVT, LATg–Staff Trainer–LAR

Revision #1 by: Name–Title–Department
Reviewed by: Name–Title–Department

Date: 8/28/2009
Date: 4/9/2012

Add additional lines for each subsequent Review/Revision
STANDARD OPERATING PROCEDURES
LABORATORY ANIMAL RESOURCES (LAR)
COLORADO STATE UNIVERSITY

LAR SOP #AHGP 031

Category: Animal Husbandry—General Procedures

Title: Animal Welfare Checks

Approved by: Lon Kendall DVM, PhD—Director—LAR

Signature: [Signature]

Effective Date: 12/15/2009

Persons Responsible
LAR Animal Care staff
LAR Veterinary and Technical Services staff

Purpose
To describe procedures for performing daily animal observation to meet or exceed State and Federal regulations and guidelines as outlined in the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals.

Safety Considerations
NOTE: THE PI IS RESPONSIBLE FOR THE SAFETY AND TRAINING OF THEIR LABORATORY PERSONNEL.

Procedure
Daily animal husbandry services are required, including weekends and holidays. Duties are variable from day to day but must include, at a minimum, daily observation of animals to verify general health and wellbeing and to assure intact, functional housing and access to adequate supplies of food and water.

Animals found moribund or in life threatening circumstances (Rodent Behavioral Score C or D, see Appendix—Rodent Behavioral Scoring) must be reported immediately, in person or by telephone, to the LAR “On Call” Veterinarian at (970) 566-3414.

1. Enter the building, animal containment facility and the animal room (see facility specific Entry/Exit SOPs).

2. Consult the Room Red Book to identify current needs, concerns and room specific instructions (see LAR SOP AHGP 044—Room Red Book).

3. Observe all animals daily for signs of illness, injury or death (see Appendix—Rodent Behavioral Scoring). In addition, observe breeder animals, if present, for any remarkable events, e.g., evidence of parturition or related complications, etc.

3.1 Place a Clinical Observation Card in the cage card holder of cages with sick and/or injured animals. Include the cage card number, the investigator, the date reported, the technician ID and a brief description of the problem.
3.2 Report sick, injured or dead animals to the LAR Veterinary and Technical Services department in the LAR Animal Medical Records System (see LAR SOP AHGP 031—Animal Medical Records).

3.3 Report and document any remarkable events in Breeder cages on Breeder Cage Cards, in Breeder Notebooks and in the Communication Log in the Room Red Book and, as required, in study specific notebooks (see LAR SOP AHSS 525—Breeding and Weaning).

3.4 Process animal carcasses as required (see LAR SOP AHGP 035—Disposition of Animal Carcasses).

4. Assure adequate supplies of food and water in every cage, every day.

4.1 Assure at least a 24 hour supply, or enough to reach the next scheduled cage/bottle change (whichever comes first).

4.2 Anticipate weekends and holidays and assure adequate supplies to reach the next regular workday.

5. Verify that basic facility functions, e.g. air handling, lighting, etc., and any mechanized caging systems are operational. Report any malfunctions to supervisors and/or facility managers.

6. On cage changing days, the Animal Welfare Check may be performed concurrently with the cage changing process.

7. Complete documentation for the day.

8. Exit the animal room, the animal containment facility and the building (see facility specific Entry/Exit SOPs).

Appendix

- Rodent Behavioral Scoring
- Clinical Observation Card

Author: Steven K. Cary BS, LATg—Animal Care Specialist—LAR Date: 12/15/2009
Reviewed by: Rick Heimbichner LAT—Animal Care Supervisor—LAR Date: 12/15/2009

Revision #1 by: Name—Title—Department Date:
Reviewed by: Name—Title—Department Date:

Add additional lines for each subsequent Review/Revision
Colorado State University Laboratory Animal Resources

Rodent Behavioral Scoring

- Normal, healthy rodent
- Awake when person is in room (except hamsters)
- Well-groomed (clean, smooth coat)
- Explores environment or observes
- Builds/repairs nest
- Normal posture and gate with flat back and no limping, hopping, staggering, or dragging/carrying of limbs
- Readily eats food and drinks water
- Facial expressions: normal with no bulging to cheeks or face, whiskers and ears forward and alert

Note: Normal behavior may be accompanied by conditions which should be reported (e.g. skin trauma, eye lesions).

- Rodent with slight or subtle behavioral changes
- May not groom adequately and coat may be a little oily or rough
- May have porphyrin staining on the coat indicating stress (rats)
- Eating and drinking adequately, but less active than normal
- May rest in "hunched" position (but this is transitory)
- Less interest in environment; other animals or people
- May have gait abnormalities or other signs of trauma/illness (e.g. nasal or ocular discharge, rectal prolapse)
- Facial expressions: mild cheek and facial bulge, eyes squinting, ears and whiskers may be held closer to head

Note: This behavior may be the result of experimental manipulation or illness

- Rodent definitely not acting normally
- Reluctant to move
- Rests upright with the back hunched (sick rodent posture)
- Back stays hunched when animal moves
- Animal will move if prodded with finger
- Not eating or drinking normally
- Poorly groomed (haircoat is oily, dirty and stands on end)
- Facial expressions: cheek and facial bulge is significant, eyes squinting or closed, ears and whiskers may be held close to head
- Notify veterinary staff immediately

Note: There should be a time limit in the protocol for animals at Score C; otherwise, veterinary services will determine if the animals should be euthanized.

- Rodent is MORIBUND (near death and will not recover)
- Animal is either hunched in sick rodent posture or lying on its side
- Only sign of life is breathing which is shallow and either slow or rapid
- Eyes sunken or closed
- If prodded, animal will only respond minimally
- May feel cool to touch
- PI should be notified that euthanasia must be performed
- Notify veterinary staff immediately

Persons Responsible
LAR Animal Care staff
LAR Veterinary and Technical Services staff
Investigator staff using rodents in shoe box caging

Purpose
To provide a course of action to mitigate the adverse effects of a cage flood and to alert both LAR Veterinary and Technical Services staff and Investigator staff.

Safety Considerations
PRINCIPAL INVESTIGATORS (PI) ARE RESPONSIBLE FOR THE SAFETY AND TRAINING OF THEIR STAFF

Procedure
Malfunctions of the water bottle based animal watering system (bottle, stopper, sipper tube) can result in the leakage of water into shoe box caging systems. The resulting cage flood can significantly compromise animal health and wellbeing. Cage flood severity is variable, dependent on the amount of water available and the absorbent capacity of the cage bedding material.

1. Observe all animals daily for signs of illness, injury or death, including cage floods (see LAR SOP #AHGP 031 Animal Welfare Checks).

2. Identify flooded cages.

   2.1 Remove and process any dead animal carcasses (see LAR SOP #AHGP 035 Disposition of Animal Carcasses).

   2.2 Change the cage if live animals are present. Include a small amount of food on the bottom of the cage, appropriate enrichment, and a new water bottle (see LAR SOP—Species Specific Husbandry)

      • Replace the dry cage onto the rack if the animals are dry or damp but otherwise appear to be in good condition, Behavior Score A or B (see Appendix—Rodent Behavioral Scoring).

      • Proceed as follows if the animals are thoroughly wet and cold, or otherwise appear to be in poor condition, Behavior Score C or D.

         o Gently dry the animal hair coats, as much as possible, using absorbent materials, i.e. napkins or paper towels.

         o Place a heating pad, adjusted to the lowest temperature setting, in the Biosafety Cabinet (BSC)/change station.
- Position the dry cage so that one half of the floor space is on the heating pad and one half is not. This will ensure that the animals have the freedom to move to a cooler area if they become too warm.
- Leave the cage on the heating pad overnight.
- Replace the cage onto the rack the following day.

- Notify investigator staff and LAR Veterinary and Technical Services staff by creating a case in the Animal Medical Records (AMR) system following ALL cage floods, regardless of severity or Behavior Score (see LAR SOP #AHGP 036 Animal Medical Records).

2.3 Attempt to diagnose the cause of the water bottle malfunction. Proper function of the water bottle depends on vacuum present inside the bottle and being maintained by surface tension of the water across the sipper tube opening. Any breach in that system will result in a leak and subsequent cage flood.

- Any crack or hole, no matter how small, in the water bottle.
- Any defect in the stopper or water bottle mouth that would prevent proper seating of the stopper/sipper tube.
- Failure to properly seat the stopper/sipper tube.
- Any compromise of the sipper tube that could interrupt the surface tension of the water across the sipper tube opening, including physical damage (tube bent or crushed) or contamination by a foreign object.

2.4 Remove damaged equipment from circulation and submit to the Cage Wash Manager for assessment, inventory adjustment and disposal.

2.5 Monitor the animals for 24 hours to identify any latent adverse effects or as recommended by the LAR Veterinary and Technical Services Staff.

Appendix
- Rodent Behavioral Scoring

| Author: Steven K. Cary  BS, LATg—Animal Care Specialist—LAR | Date: 11/6/2009 |
| Reviewed by: Jessica Ayers  DVM, DACLAM—Associate Director—LAR | Date: 3/12/2014 |

| Revision #1 by: Name—Title—Department | Date: |
| Reviewed by: Name—Title—Department | Date: |
Colorado State University Laboratory Animal Resources

Rodent Behavioral Scoring

- Normal, healthy rodent
- Awake when person is in room (except hamsters)
- Well-groomed (clean, smooth coat)
- Explores environment or observes
- Builds/repairs nest
- Normal posture and gate with flat back and no limping, hopping, staggering, or dragging/carrying of limbs
- Readily eats food and drinks water
- Facial expressions: normal with no bulging to cheeks or face, whiskers and ears forward and alert

Note: Normal behavior may be accompanied by conditions which should be reported (e.g. skin trauma, eye lesions).

- Rodent with slighter subtle behavioral changes
- May not groom adequately and coat may be a little oily or rough
- May have porphyrin staining on the coat indicating stress (rats)
- Eating and drinking adequately, but less active than normal
- May rest in "hunched" position (but this is transitory)
- Less interest in environment, other animals or people
- May have gait abnormalities or other signs of trauma/illness (e.g. nasal or ocular discharge, rectal prolapse)
- Facial expressions: mild cheek and facial bulge, eyes squinting, ears and whiskers may be held closer to head

Note: This behavior may be the result of experimental manipulation or illness

- Rodent definitely not acting normally
- Reluctant to move
- Rests upright with the back hunched (sick rodent posture)
- Back stays hunched when animal moves
- Animal will move if prodded with finger
- Not eating or drinking normally
- Poorly groomed (haircoat is oily, dirty and stands on end)
- Facial expressions: cheek and facial bulge is significant, eyes squinting or closed, ears and whiskers may be held closer to head
- Notify veterinary staff immediately

Note: There should be a time limit in the protocol for animals at Score C, otherwise veterinary services will determine if the animals should be euthanized.

- Rodent is MORIBUND (near death and will not recover)
- Animal is either hunched in sick rodent posture or lying on its side
- Only sign of life is breathing which is shallow and either slow or rapid
- Eyes sunken or closed
- If prodded, animal will only respond minimally
- May feel cool to touch
- PI should be notified that euthanasia must be performed
- Notify veterinary staff immediately

PERSONS RESPONSIBLE
Investigator staff
LAR Veterinary and Technical Services staff
LAR Animal Care staff

PURPOSE
To describe the procedure for reporting and documenting incidences of morbidity, including veterinary care, and/or mortality of laboratory animals.

SAFETY CONSIDERATIONS
PRINCIPAL INVESTIGATORS (PI) ARE RESPONSIBLE FOR THE SAFETY AND TRAINING OF THEIR STAFF

PROCEDURE
All animals are observed daily for signs of illness, injury or death. All incidences of morbidity (illness and injury) and mortality (death) of laboratory animals are reported to the LAR Veterinary and Technical Services department electronically, in the LAR Animal Medical Records System (AMR) or by using hard copy Morbidity or Mortality Report Forms (M&M), as needed.

Animals found moribund or in life threatening circumstances (Rodent Behavioral Score C or D, see Appendix—Rodent Behavioral Scoring) must be reported immediately, in person or by telephone, to the LAR "On Call" Veterinarian at (970) 566-3414

Electronic LAR Animal Medical Records System

1. Identify sick, injured or dead animals.
   1.1 Place a Clinical Observation Card (see Appendix) in the cage card holder of cages with sick and/or injured animals. Enter the investigator name, the cage card number, the date reported, the technician ID and a brief description of the problem.
   1.2 Amend rodent cage cards to reflect observed mortalities.

   - Adjust the cage card population count to reflect the number of remaining animals.
   - Note the number of animals found dead (include individual animal ID # if possible), the current date and your initials.
   - Enter the current date as the “Date Terminated” if all animals represented by the cage card are deceased.

   1.3 Process animal carcasses (see LAR SOP #AHGP 035—Disposition of Animal Carcasses).
1.4 Submit “terminated” cage cards or cage card numbers to the LAR Main Campus or Foothills Campus Administrative Offices.

2. Note all pertinent cage card information and observations for later entry into the AMR System.

3. Access the AMR on line. From the LAR website:
   
   3.1 Select “Research Portal (restricted)”
   3.2 Enter your eID and password to the Campus Administrative Portal (CAP).
   3.3 Select “Lab Animal Resources”
   3.4 Select “Animal Medical Records System”

4. Select “Submit a Case”

5. Enter the cage card number of the animal(s). The animal management software system (e.g. Granite) will automatically populate a portion of the form with cage card information.

6. Enter additional information as required.
   
   6.1 Record any additional cage card information not automatically downloaded from the animal management software system.
   6.2 Complete the “Case Information” fields as completely as possible.
   6.3 Record, in layman terms, your personal observations, including a Rodent Behavior Score (see Appendix—Rodent Behavioral Scoring).

7. Select “Create a New Case”. The AMR will create and assign a unique “Case Number”.

8. Within one day of receipt of a reported morbidity, LAR Veterinary and Technical Services staff will:
   
   8.1 Perform triage.
      
      - Assess the animal(s).
      - Enter the Vet Exam Date and the Vet ID on the Clinical Observation Card.
      - Notify the investigator.
      - Establish an action plan, with investigator input, including any potential medical treatment, as required.

   8.2 Enter required details of the action plan on the Clinical Observation Card.
   8.3 Assign responsibility for ongoing health monitoring and/or medical treatments to one or more of the following.
      
      - LAR Animal Care staff (AC)
      - LAR Veterinarians (V)
      - LAR Technical Services staff (TS)
      - Investigator staff (PI)

   8.4 The responsible party documents performance of the action plan on the Clinical Observation Card and, as required, in Study Specific Notebooks.
   8.5 Veterinary and Technical Services staff retrieve, scan and electronically attach completed Clinical Observation Cards to the AMR.

9. Within one day of receipt of a reported mortality, LAR Veterinary and Technical Services staff will notify the investigator and, if possible and/or required, perform a necropsy.

10. Select “Review/Edit a Case” to monitor progress or provide updates to established Medical Cases.
11. Animal medical records will be retained by LAR for at least one year for mice and rats and five years beyond final disposition for USDA covered species.

Alternate Reporting Options (Investigator staff and others without LAR Intranet access)

12. Report non-emergent M&Ms (Rodent Behavioral Score A or B) via an entry in the Communication Log of the Room Red Book (see LAR SOP #AHGP 044 Room Red Book). Within 24 hours, LAR Animal Care staff will:

12.1 Identify those entries during daily animal welfare checks (see LAR SOP #AHGP 031 Animal Welfare Checks).
12.2 Report them in the AMR as described above.

OR


13.1 Proceed as described in 1 above.
13.2 Enter all pertinent information onto the appropriate hard copy M&M Form.
13.3 Submit completed, hard copy M&M Report Forms to the LAR Veterinary and Technical Services department.
   - Deliver hard copies to the Technical Services Office at the Painter Center.
   - Address electronically transferred forms (fax, scan, e-mail) to the LAR Veterinary Technician.
13.4 LAR Technical Services staff will enter information from the hard copy M&M Report Forms into the AMR system and proceed as described above.

Biocontainment Barrier Expectations

14. Investigators in ABSL-3 facilities (the Infectious Disease Research Center [IDRC] and the Painter Center B wing and D102 suite) require immediate notification of Morbidities and Mortalities. In addition to the procedures described above, Animal Care staff must:

14.1 Consult the Room Specific Instructions sheet, the Room Red Book, direct interaction with investigators and LAR Animal Care Supervisors for specific requirements.
14.2 Notify investigators, as agreed, in person, by telephone or by e-mail.

Appendix
- Morbidity Form
- Mortality Form
- Clinical Observation Card
- Rodent Behavioral Scoring
<table>
<thead>
<tr>
<th>Author: Steven K. Cary BS, LATg--Animal Care Specialist--LAR</th>
<th>Date: 5/15/2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reviewed by: Jessica Ayers DVM, DACLAM--Associate Director--LAR</td>
<td>Date: 10/8/2014</td>
</tr>
<tr>
<td>Revision #1 by: Name--Title--Department</td>
<td>Date:</td>
</tr>
<tr>
<td>Reviewed by: Name--Title--Department</td>
<td>Date:</td>
</tr>
</tbody>
</table>

Add additional lines for each subsequent Review/Revision
# Morbidity Report

- Date: ____________________________
- CC#: ____________________________
- PI: ________________________________
- Protocol #: _________________________
- Contact: ___________________________
- Phone: ____________________________
- Species: ___________________________
- Strain: ____________________________
- Gender: ____________________________
- Rec. Date: _________________________
- DOB: ______________________________
- Vendor: ____________________________
- Animal ID#: _______________________
- Building/Room/Location: ____________
- # of animals affected: ______________
- Additional CC Info: ________________
- Comments: _________________________
- __________________________________________________________________________
- Caretaker: _________________________

Exam Findings/Rule Outs:

Treatment:

<table>
<thead>
<tr>
<th>Date</th>
<th>AM</th>
<th>PM</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Veterinarian/Date: ____________________________
# Mortality Report

- **Date:**
- **CC#:**
- **PI:**
- **Protocol #:**
- **Contact:**
- **Phone:**
- **Species:**
- **Strain:**
- **Gender:**
- **Rec. Date:**
- **DOB:**
- **Vendor:**
- **Animal ID#:**

- **Building/Room/Location:**
- **# of animals affected:**
- **Additional CC Info:**
- **Comments:**
- **Caretaker:**

---

**Discard:**

**Necropsy:**

**Gross:**

**Histo Case#:**

**Gross Findings:**

<table>
<thead>
<tr>
<th>System</th>
<th>No lesions</th>
<th>Lesions found</th>
<th>Not examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>General condition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Integ/Subcutis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphohemopoietic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digestive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genitourinary</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocrine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNS/SS</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**Veterinarian/Date:**

**PI Notified:**
Colorado State University Laboratory Animal Resources

Rodent Behavioral Scoring

A. Normal, healthy rodent
   - Awake when person is in room (except hamsters)
   - Well-groomed (clean, smooth coat)
   - Explores environment or observes
   - Builds/repairs nest
   - Normal posture and gate with flat back and no limping, hopping, staggering, or dragging/carrying of limbs
   - Readily eats food and drinks water
   - Facial expressions: normal with no bulging to cheeks or face, whiskers and ears forward and alert
   - Note: Normal behavior may be accompanied by conditions which should be reported (e.g. skin trauma, eye lesions).

B. Rodent with slight or subtle behavioral changes
   - May not groom adequately and coat may be a little oily or rough
   - May have porphyrin staining on the coat indicating stress (rats)
   - Eating and drinking adequately, but less active than normal
   - May rest in "hunched" position (but this is transitory)
   - Less interest in environment, other animals or people
   - May have gait abnormalities or other signs of trauma/illness (e.g. nasal or ocular discharge, rectal prolapse)
   - Facial expressions: mild cheek and facial bulge, eyes squinting, ears and whiskers may be held closer to head
   - Note: This behavior may be the result of experimental manipulation or illness

C. Rodent definitely not acting normally
   - Reluctant to move
   - Rests upright with the back hunched (sick rodent posture)
   - Back stays hunched when animal moves
   - Animal will move if prodded with finger
   - Not eating or drinking normally
   - Poorly groomed (haircoat is oily, dirty and stands on end)
   - Facial expressions: cheek and facial bulge is significant, eyes squinting or closed, ears and whiskers may be held close to head
   - Notify veterinary staff immediately
   - Note: There should be a time limit in the protocol for animals at Score C, otherwise veterinary services will determine if the animals should be euthanized.

D. Rodent is MORIBUND (near death and will not recover)
   - Animal is either hunched in sick rodent posture or lying on its side
   - Only sign of life is breathing which is shallow and either slow or rapid
   - Eyes sunken or closed
   - If prodded, animal will only respond minimally
   - May feel cool to touch
   - PI should be notified that euthanasia must be performed
   - Notify veterinary staff immediately

StANDARD OPERATING PROCEDURES
LABORATORY ANIMAL RESOURCES (LAR)
COLORADO STATE UNIVERSITY

LAR SOP #AHGP 034

Category: Animal Husbandry—General Procedures
Title: Carbon Dioxide (CO₂) Euthanasia of Rodents
Approved by: Lon Kendall DVM, PhD—Director—LAR
Signature: [Signature]
Effective Date: 7/12/2010

Persons Responsible
Investigator staff
LAR Veterinary and Technical Services staff
LAR Animal Care staff

Purpose
To standardize “best practices” procedures for the humane euthanasia of laboratory rodents as recommended by the 2006 Newcastle Consensus Meeting on Carbon Dioxide Euthanasia of Laboratory Animals and the AVMA Panel on Euthanasia.

Safety Considerations
NOTE: THE PI IS RESPONSIBLE FOR THE SAFETY AND TRAINING OF THEIR LABORATORY PERSONNEL.

Procedure
Exposure to CO₂ gas is a widely accepted method of rodent euthanasia. General consensus has established that the most humane procedure requires the gradual delivery of CO₂ gas using a precision flow meter.

1. Obtain a euthanasia chamber, preferably the animal’s home cage.
   1.1 Do not overcrowd.
   1.2 Do not combine multiple species.

2. Obtain and assemble a euthanasia chamber cover, a flow meter and the necessary hoses to attach those items to a CO₂ source (see Appendix—Directions for CO₂ Euthanasia of Rodents).

3. Place the euthanasia cover over the euthanasia chamber.

4. Turn the CO₂ source to the “On” position.
   4.1 Some locations rely on portable CO₂ tanks, with additional gauges.
   4.2 Some locations rely on taps supplied from a central location.

5. Adjust the flow meter control to introduce 100% CO₂ to the euthanasia chamber to result in the replacement of 20% of the cage volume per minute. Flow rates for common LAR cages are:
   5.1 Thoren #1 Mouse cage: 5.8L, flow rate of 1.2 L/min
   5.2 Thoren #9 Mouse cage: 6.5L, flow rate of 1.3 L/min
   5.3 Optimice cages: 6.2L, flow rate of 1.2 L/min
   5.4 Tecniplast SealSafe (blue & green lines): 7.6L, flow rate of 1.5L/min
5.5 Tecniplast IsoCage (orange line): 7.1L, flow rate of 1.4L/min
5.6 Deep Rat Cage (e.g. Ancare R20): 26.2L, flow rate of 5.2L/min
5.7 Calculate flow rates for other cage/chamber sizes (see Appendix—Directions for CO2 Euthanasia of Rodents).

6. Increase the flow rate to 3-4 times when unconsciousness (approximately 3-4 minutes) has been induced in the animal(s).

7. Maintain the flow rate for a minimum of one minute beyond the apparent clinical death of the animal(s).

8. Turn the flow meter and the CO2 source to the “Off” position.

9. Confirm the clinical death of the animal(s).
   - Observe the animals for a minimum of five minutes following apparent clinical death.
   - Perform cervical dislocation or bilateral thoracotomy.


11. Return the euthanasia chamber cover, the flow meter and hoses to storage.

12. Transport soiled caging and equipment to the appropriate “dirty” equipment storage location or to the Dirty Side of the Cage Wash facility (see facility specific SOPs).

Appendix

- CO2 Euthanasia “Best Practices” Guidelines
- Directions for CO2 Euthanasia of Rodents

| Author: Steven K. Cary BS, LATg—Animal Care Specialist—LAR | Date: 9/28/2009 |
| Reviewed by: Elizabeth Magden DVM—Resident Veterinarian—LAR | Date: 7/12/2010 |

Revision #1 by: Name—Title—Department
Reviewed by: Name—Title—Department

Add additional lines for each subsequent Review/Revision
CO₂ Euthanasia Best Practices Guidelines

Rationale: This project was initiated in order to 1. Evaluate the literature regarding use of CO₂ euthanasia in rodents, 2. Evaluate current CSU CO₂ euthanasia practices for mice in a variety of labs currently using the technique, and 3. Develop a recommended protocol for use of CO₂ euthanasia, if feasible, to present to the CSU IACUC for consideration and adoption. This report was researched and prepared by Dr. Elizabeth Magden in consultation with other LAR veterinarians, Dr. Ray Whalen, and RICRO staff.

Literature Review

Over ten manuscripts published between 1993 and 2008 that provided original research on use of CO₂ euthanasia were reviewed. The findings of these reports were consistent with the recommendations made at the Newcastle Consensus Meeting on Carbon Dioxide Euthanasia of Laboratory Animals (Hawkins et al.) This report was generated by a panel of experts from both sides of the CO₂ euthanasia debate convened in 2006. The group identified a list of problems associated with CO₂ euthanasia, but also acknowledged the advantages of CO₂ as a euthanasia technique. These include: (1) CO₂ is a rapid depressant, (2) CO₂ has well-established anesthetic and analgesic effects, (3) it is readily available and relatively safely and easily administered, (4) CO₂ does not leave any tissue residues, (5) CO₂ does not increase serum corticosterone concentrations. The disadvantages of CO₂ use are: (1) it is not ideal for all species (reptiles, rodent neonates, fish, diving mammals), (2) lower concentrations (<80%) may cause pulmonary lesions, and, most importantly, (3) administration of high concentrations may be distressful to the animals being euthanized (AVMA Guidelines 2007).

The panel agreed on the following key points:

- There is no “ideal” method of killing animals
- Both pre-filled and gradual induction techniques with CO₂ may not provide smooth euthanasia
- If the concentrations of CO₂ exceed 50%, then the animals experience 10-15 seconds of mucosal pain prior to losing consciousness
- As CO₂ concentrations rise, the animals will find a certain level aversive and experience dyspnea.

The Newcastle panel determined that it is more important to avoid or minimize pain than to induce rapid unconsciousness, therefore ultimately recommended the use of low flow gradual induction methods during CO₂ euthanasia. Specifically, the panel recommended administration of 100% CO₂ at a flow rate of 20% chamber volume per minute to produce a loss of consciousness without apparent pain. Flow rate could be increased following unconsciousness. A study conducted by Danneman et al. supports the aforementioned proposed CO₂ euthanasia protocol (1997). This report found that the CO₂ concentrations less than 70% are least likely to cause pain and/or distress. Danneman et al. also concluded that the most humane method of CO₂ euthanasia is to
gradually induce the animal with a low flow rate of CO₂, thereby avoiding the exposure of a conscious animal to CO₂ levels greater than 70%.

The Newcastle panel addressed the use of volatile anesthetic agents (such as isoflurane) as an alternative to CO₂ euthanasia during their 2006 consensus meeting. The panel agreed that sufficient research does not exist to determine the relative aversiveness or other effects on rodents when used as the sole euthanasia agent. Another potential issue with volatile gas euthanasia, is that historically CO₂ euthanasia has been used in terminal animal studies, and researchers may be adding an unwanted variable to any future post-mortem studies if alternative euthanasia methods were to be adopted. There are also health concerns for workers performing euthanasia with volatile anesthetic agents.

It has been suggested that adding a sedative or pre-anesthetic agent prior to CO₂ exposure may decrease any associated distress. A study by Hackbarth et.al. (2000) examined this theory in rats by using CO₂ euthanasia alone, or by pre-treating with acepromazine or pentobarbital. They monitored animal behavior, glucose, ACTH, and corticosterone concentrations as indicators of increased stress. They found no difference in animal distress using behavioral or hormonal changes as primary indicators, although those animals receiving an injection of pentobarbital did have higher levels of ACTH, indicating the pre-injection apparently induced more stress than CO₂ use alone.

The most recent AVMA Panel on Euthanasia supports the use of CO₂ for euthanasia in appropriate species (refer to Appendices 1 and 2 in AVMA Guidelines 2007), utilizing a compressed gas cylinder where inflow can be precisely regulated. The AVMA agrees with the Newcastle panel consensus, in that the optimal flow rate is to displace at least 20% of chamber volume per minute and to maintain gas flow for one minute following clinical death. The AVMA additionally recommends that different species should not be combined and cages should not be overcrowded during the euthanasia. The cage floor should be large enough for each animal to stand on four feet with enough space remaining to turn around and perform normal postural adjustments (Artwohl et.al. 2006).

Evaluation of Current CSU practices

Four sites were visited to evaluate CO₂ euthanasia practices. These practices varied from 100% CO₂ pre-filled chambers to a gradual induction with a CO₂ flow rate that was just barely audible. No locations visited utilized flow meters. The personnel with the most extensive experience in CO₂ euthanasia believed that gradual induction with a low flow of CO₂ seemed to be the most humane method. The variation in CO₂ euthanasia techniques across campus calls for a standardization to ensure that as a campus CSU is implementing the established most humane CO2 euthanasia techniques. Given the preceding research results regarding the use of CO₂ euthanasia, and the laboratory observations, a method was developed to consistently implement a euthanasia protocol consistent with the 2006 Newcastle Consensus Meeting on Carbon Dioxide Euthanasia of Laboratory Animals (Hawkins et.al.). With the assistance of Dr.
Thomas Johnson (Env. & Rad. Health Sciences), we obtained a flow meter to determine the gas flow rates achieved when the flow is turned on to an audibly low level. The flow rates using this audible low flow method were approximately 10 times the recommended 20% chamber volume displacement per minute. By installing a flow meter we can accurately gage the flow of CO₂ into the cages and use procedures consistent with the established best practices of CO₂ euthanasia as determined by the Newcastle panel consensus, AVMA, the report of the ACLAM task force, and various authors.

In order to determine the flow rate necessary for a 20% volume displacement, the cage volume must first be calculated. Cage volume (L) is divided by 5 (to determine 20% volume) and this is the flow rate to be used during euthanasia (in liters/minute). The inlet end of the flow meter is connected to tubing from the CO₂ canister, and the outflow end is connected to tubing leading into the euthanasia chamber.

Following development of this protocol, approximately 100 mice were euthanized using this method. These mice appeared to experience a consistently smoother induction than those euthanized without the flow meter. Over a course of three to five minutes they became ataxic and lethargic, and then unconscious prior to death. Without the use of a flow meter it has been a general observation during CO₂ euthanasia that mice cluster in the cage corners prior to death – possibly due to a panic response. This behavior was not observed with the flow meter regulating CO₂ flow. Videos were taken during the procedures that will be available for IACUC review.

Based upon these observations, LAR veterinarians recommended the IACUC adopt the following ‘best practices’ guidelines for use of CO₂ as a euthanasia agent:

1. 100% CO₂ should be gradually introduced to result in replacement of 20% chamber volume per minute to produce a loss of consciousness without apparent pain.

2. A flow meter (Dwyer instruments, VFB-65-BV flow meter, range 0.2 to 4 L/min) should be installed on every CO₂ canister in order to monitor the flow rate, and the appropriate rate calculated for the volume of the euthanasia chamber. This is done by determining the volume in the cage in liters and dividing by 5.

3. Following the induction of unconsciousness, the rate can be raised to 3-4 times the initial flow rate to accelerate the process.

4. After apparent clinical death of the animal, gas flow should be maintained for at least one minute.

5. Death should be verified via cervical dislocation or bilateral thoractomy.
6. Animal density and mixing of different cages in the euthanasia chamber should be minimized to decrease pre-euthanasia anxiety, as our empirical observations indicated this provided for a less stressful euthanasia.

References:


use of laboratory animals clarification regarding use of carbon dioxide for euthanasia of small laboratory animals.

Directions for CO₂ Euthanasia of Rodents

1. Secure a euthanasia chamber, ideally using the home cage. Animal density and mixing of different animals from separate cages in the euthanasia chamber should be minimized to decrease pre-euthanasia anxiety.

2. The volume of the euthanasia chamber (in liters) should be calculated and then divided by 5 in order to determine the appropriate CO₂ flow rate (20%).

If using one of the LAR standard cages the volumes are as follows:

- #1 Mouse Thoren cage: 5.8L, flow rate of **1.2 L/min**
- #9 Mouse Thoren cage: 6.5L, flow rate of **1.3 L/min**
- OptiMice cages: 6.2L, flow rate of **1.2 L/min**
- Tecniplast SealSafe (blue & green lines): 7.6L, flow rate of **1.5L/min**
- Tecniplast IsoCage (orange line): 7.1L, flow rate of **1.4L/min**
- Deep Rat Cage (e.g. Ancare R20): 26.2L, flow rate of **5.2L/min**

3. The cover should be placed over the euthanasia chamber/cage, and the flow meter should be checked to ensure it is in the off position. The CO₂ canister valve is then turned on (A) and the pressure gauge closest to the tank should register a pressure. The second gauge (B) should then be turned on. After both valves have been turned on, the flow meter can be adjusted to the appropriate flow rate (liters/min) in order gradually introduce 100% CO₂ to result in the replacement of 20% of the cage volume per minute. This flow rate has been shown to produce a loss of consciousness without apparent pain (see attached report, Appendix 1).

4. Following the induction of unconsciousness (this will take approximately 3-4 minutes), the CO₂ flow rate can be raised to 3-4 times the initial flow rate to accelerate the process. Following apparent clinical death of the animal, gas flow should be maintained for at least one minute.

5. Following death the flow meter and two gauges should all be turned to the off position.

6. Cervical dislocation or bilateral thoracotomy should be performed to assure the animal will not regain consciousness. Euthanized animals should be placed in a sealable bag and discarded on the lower shelves of the necropsy cooler unless there is some other need for the animals or their tissues. Biohazardous or otherwise hazardous carcasses should be discarded using procedures established for the study in question.
NOTE: Neonate rodents ("pinkies") will become anesthetized with CO₂. However, due to their high levels of fetal hemoglobin they are resistant to death via CO₂. Cervical dislocation or decapitation (following CO₂ narcotization) is the best method of euthanasia for these neonates.
Persons Responsible
Investigator staff
LAR Veterinary and Technical Services staff
LAR Animal Care staff

Purpose
To describe the storage and disposal of animal carcasses in order to assure collection of terminal samples, as required, including organs, tissues and fluids, and to assure both biosafety and biosecurity in the disposal of the remains.

Safety Considerations
NOTE: THE PI IS RESPONSIBLE FOR THE SAFETY AND TRAINING OF THEIR LABORATORY PERSONNEL.

Procedure
Animal carcasses are generated intentionally, by humane euthanasia, and spontaneously in the laboratory setting. Individual investigators may request post mortem access and/or additional post mortem services. Check the Room Specific Instructions sheet for investigator specific instructions concerning the handling and processing of animal carcasses.

1. Animals maintained in conventional housing.

   1.1 Place the carcass(es) in a plastic bag.

       • Use ziplock type bags for small species, trash bags for larger species.
       • Avoid overfilling bags regardless of species or bag size.

   1.2 Label the bag using a permanent marker.

       • Include at least the following Infectious Disease Research Center (IDRC) required information:

           o Cage card number
           o Principle Investigator
           o Date
           o Your first initial and full last name
           o Indicate whether to “save” or “trash”

       • Include additional LAR information, as needed
1.3 Place the bagged carcass(es) in short term cold storage (refrigerator) separating bags marked “save” from bags marked “trash” if possible, pending necropsy and/or tissue collection, as required, and/or disposal.

1.4 Periodically consolidate “trash” and other disposable, processed carcasses and tissues into larger “red” bags (for convenience of handling) and place them in long term cold storage (freezer) pending incineration or tissue digestion.

1.5 Submit an LAR Work Order Form (see LAR SOP—Work Order Form) to arrange for the transport of animal carcasses/tissues to the incinerator at the Foothills Research Campus or to the Tissue Digester at the Veterinary Medical Center.

1.6 Carcasses of “clean” animals, those having not been exposed to potentially dangerous pathogens or chemicals, may be saved, frozen, and used for teaching purposes or donated as food items to organizations maintaining populations of reptiles and/or predatory birds and mammals.

2. Non-Select Agent Animals Maintained at ABSL-3

2.1 All open cage/culture animal husbandry duties, including the processing of animal carcasses, must be performed in the Biosafety Cabinet (BSC) (see LAR SOP Biosafety Cabinets/Change Stations and/or IDRC SOP—Biosafety Cabinet)

2.2 Pre-treat the interiors of two autoclavable bags with a pathogen specific disinfectant unless directed otherwise by the investigator.

2.3 Place the carcass(es) in the first autoclavable bag. Seal the bag (tie, twist tie or tape) and place it in the second autoclavable bag. Seal with autoclave indicator tape. Avoid overfilling the bags.

2.4 Label the bag using a permanent marker and autoclave indicator tape as a writing surface.

   - Include at least the following IDRC required information:
     
     o Cage card number
     o Principle Investigator
     o Date
     o Your first initial and full last name
     o Indicate whether to “save” or “trash”

   - Include additional LAR information, as needed.

     o Room Number
     o Pathogen, if applicable

2.5 Spray the bagged carcasses with a pathogen specific disinfectant before removal from the BSC and again before removal from the animal room.

2.6 Place the bagged carcasses in cold storage.

   - Refrigerate or freeze, per investigator request, bags marked “save”.
   - Freeze bags marked “trash”.

2.7 Periodically consolidate “trash” and other disposable, processed carcasses and tissues into larger autoclavable bags (for convenience of handling) and autoclave out of the ABSL-3 containment barrier.

2.8 Maintain autoclaved carcasses and tissues in cold storage (frozen) until disposal by incineration or digestion.
3. Select Agent Animals Maintained at ABSL-3

3.1 Proceed as described in 2.1-2.5 above.

3.2 Select Agent animal carcasses MUST be stored within the Select Agent approved animal room until removal for autoclaving/incineration.

- Place the bagged carcasses in room specific cold storage, when a room specific cold storage option is available.

OR

- Retain the bagged carcasses in the BSC when a room specific cold storage option is not available.

3.3 Proceed as described in 2.5 and 2.7-2.8 above for removal, decontamination and disposal of Select Agent animal carcasses.
PERSONS RESPONSIBLE
All LAR Users

PURPOSE
The Room Red Book provides a central location for LAR animal room documentation, including, but not limited to, the Communication Log, Morbidity/Mortality (M&M) reporting and tracking information, and hard copy Cage Card Request Forms.

SAFETY CONSIDERATIONS
PRINCIPAL INVESTIGATORS (PI) ARE RESPONSIBLE FOR THE SAFETY AND TRAINING OF THEIR STAFF

PROCEDURE
LAR Animal Care Technicians must consult the Room Red Book daily to access current information pertinent to each animal room.

1. Communication Log (see Appendix)
   1.1 Use the log as an open channel of communication between any and all combinations of investigator and LAR Animal Care, Veterinary and Technical Services staffs, including:
      • Principle Investigators (Pls)
      • Research Technicians
      • LAR Animal Care Technicians
      • LAR Veterinarians
      • LAR Technical Services Technicians
   1.2 Maintain the log on a single laminated page.
   1.3 Make entries using the provided wet erase marker.
   1.4 Record events not already documented on the Animal Husbandry and Room Monitoring Sheet, as needed.
   1.5 Identify M&M information entered by investigator staff and submit in the Animal Medical Records (AMR) system as needed (see LAR SOP #AHGP 033 Animal Medical Records).
   1.6 Wipe the log clean when filled or as needed.
   1.7 Carry forward information regarding any ongoing issues or concerns, as needed.

2. Miscellaneous Laminated Documents.
   2.1 LAR Contact List
   2.2 Rodent Behavioral Scoring Sheet (see LAR SOP #AHGP 031 Animal Welfare Checks)
2.3 Animal Room Temperature and Humidity Guidelines (see LAR SOP #QC 022 Animal Room Temperature and Humidity Guidelines).
2.4 Common Medical Abbreviations Sheet

3. Cage Card Request Forms (see LAR SOP #LF 002 Cage Card Request Form).
   3.1 Record cage card information for later entry into the electronic Cage Card Request Form.

   OR

   3.2 Submit hard copy Cage Card Request Forms directly to LAR Animal Care Supervisors or Technical Services Staff.

   4.1 Record information regarding sick, injured or dead animals for later entry into the electronic LAR AMR system by LAR Animal Care Staff with LAR intranet access.

   OR

   4.2 Alternatively, staff and investigators without LAR intranet access may use these hard copy forms to report sick, injured or dead animals directly to LAR Veterinary and Technical Services staff (see LAR SOP #AHGP 033 Animal Medical Records).

   THEN

   4.3 Use a hard copy of the Morbidity form in the animal room to document ongoing medical treatments, as needed.

5. Pocket insert(s).
   5.1 Organize and maintain additional room specific documents, e.g., medical treatment sheets, etc., as needed.
   5.2 Remove and archive or discard outdated and/or unused documents, as appropriate.

APPENDIX:
• Communication Log

| Author: Steven K. Cary BS, LATg—Animal Care Specialist—LAR | Date: 12/19/2013 |
| Reviewed by: Jessica Ayers DVM, DACLAM—Associate Director—LAR | Date: 8/11/2014 |

Revision #1 by: Name—Title—Department
Reviewed by: Name—Title—Department

Add additional lines for each subsequent Review/Revision
**COMMUNICATION LOG**

<table>
<thead>
<tr>
<th>DATE</th>
<th>ANIMAL ROOM COMMUNICATION LOG</th>
<th>NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**PLEASE LOG ANYTHING YOU NOTICE. THIS IS FOR YOU AND THE INVESTIGATOR TO KEEP IN TOUCH WITH ANIMAL MONITORING**
Persons Responsible
Investigator staff
LAR Veterinary and Technical Services staff
LAR Animal Care staff

Purpose
To provide identification of laboratory animals and/or cages of laboratory animals to meet or exceed State and Federal regulations and guidelines as outlined in the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals.

Safety Considerations
PRINCIPAL INVESTIGATORS (PI) ARE RESPONSIBLE FOR THE SAFETY AND TRAINING OF THEIR STAFF

Procedure
All laboratory and teaching animals must be positively identified by, at least, a cage card. In addition, some species, e.g. dogs, cats, livestock, etc., require individual, “on the animal”, identification to meet regulatory standards. Finally, investigators may choose, at additional expense, to apply “on the animal” identification to any species of animal to meet study needs.

1. Cage Cards (uniquely numbered, computer generated) (see Appendix)

   1.1 Cage cards are generated automatically, when linked to Animal Orders, in the animal management software system (i.e. Granite) or manually, within the software system, as needed.

   1.2 Cage cards are generated by the cage for several species, including, but not necessarily limited to, most small rodents and juvenile chickens, and by the individual animal for all other species.

   1.3 Required cage card information includes, at least:

      - The name of the Principle Investigator (PI)
      - The name of the Contact Person (if different)
      - Contact information, i.e. phone number
      - The Protocol Number issued by the institutional Animal Care and Use Committee (IACUC)
      - The species/strain of animal(s)
      - The source of the animal(s)
      - Pertinent dates, e.g. Expected Shipment Date.

   1.4 Additional, pre-printed cage card information may also include:
• The sex of the animal(s)
• Date of birth
• Individual animal identification number(s)
• Requisition number

1.5 Remaining space on the cage card is available for manual data entry by both investigators and Animal Care Technicians.

• The cage population count is entered and updated, as needed, in an upper corner of the cage card.
• Technicians enter the date, the number of individuals involved, any available ID number(s), and their initials to document deceased animals.
• Investigators may enter a variety of study related data.

2. Temporary Cage Cards

2.1 Temporary Cage Cards are generated, as needed, by investigator staff or Animal Care staff, to temporarily identify animals or cages of animals not yet assigned a Permanent Cage Card. This includes, but is not necessarily limited to:

• Cage separations
• Weaning of breeding colony progeny
• Delivery of unexpected animals

2.2 Pre-printed Temporary Cage Cards, in several formats depending on specific needs, are available from LAR administrative offices.
2.3 Enter all requested information and place the Temporary Cage Card(s) on the appropriate cage(s).
2.4 Submit a Cage Card Request Form (see LAR SOP—Cage Card Request Form) to an LAR Animal Care Supervisor for production of Permanent Cage Cards.

• Submit requests to the Main Campus Animal Care Supervisor for animals held at the Painter Center, Main Campus satellites and the Veterinary Teaching Hospital (VTH).
• Submit requests to the Foothills Campus Animal Care Supervisor for animals held in all Foothills Campus locations.

2.5 Replace the Temporary Cage Card(s) with the corresponding Permanent Cage Card(s) as soon as possible.

• Accurately transfer any cage card information not already present from the Temporary Cage Card to the Permanent Cage Card.
• Retain the Temporary Cage Card behind the Permanent Cage Card in the cage card holder when possible.

3. Individual “On the Animal” Identification

3.1 Temporary tags, usually metal or plastic, applied to the animal with a removable collar.
3.2 Semi-permanent tags, e.g. ear tags intended to be long term or permanent but with the potential for traumatic loss.
3.3 Ear notches/punches, primarily for rodents (see Appendix—Ear Punch Identification Code).
3.4 Leg/neck bands, primarily for fowl.
3.5 Permanent tattoos, usually in the ear, on the flank or on the tail.
3.6 Microchips, usually installed subcutaneously, on the back, between the shoulder blades.
- Microchip identification is not provided by LAR
- Individual investigators are responsible for microchip identification as needed.

Appendix
- Cage Card
- Temporary Cage Card
- Ear Punch Identification Code

| Author: Steven K. Cary BS, LATg—Animal Care Specialist—LAR | Date: 1/15/2014 |
| Reviewed by: Jessica Ayers DVM, DAACLAM—Associate Director—LAR | Date: 3/12/2014 |

Revision #1 by: Name—Title—Department
Reviewed by: Name—Title—Department

Add additional lines for each subsequent Review/Revision

---

**TEMPORARY CAGE CARD**

PI Name: ________________
Protocol#: ________________
D.O.B. ________________
Wean/Sep Date: ________________
From CC# ________________
Strain: ________________
Qty/Sex: ________________
CC Requested by: ________________
Contact #: ________________

Cage Card Number: 115347
PI: Kesel, Martha Lynne
Protocol Number: 11-2425A (2)
Contact/Phone: Elisa French 1-1045
Cost Center: Mice-10 day
Species: Mouse
S/S/B: ICR
Gender: Female
Animal Birth Date: ________________
Animal ID: ________________
Vendor Name: Harlan
Shipment Expected Date: 11/14/2011
Requisition Number: 27655

DATE TERMINATED: 5/6/12
AIDLA
Figure 4.4  Ear punch identification code.
PERSONS RESPONSIBLE
LAR Animal Care staff
LAR Veterinary and Technical Services staff

PURPOSE
To establish a standard method of assigning alpha numeric identification to animal cage racks and individual animal cages to facilitate the transfer of information between Animal Care staff, Veterinary and Technical Services staff and investigator staff.

SAFETY CONSIDERATIONS
NOTE: THE PI IS RESPONSIBLE FOR THE SAFETY AND TRAINING OF THEIR LABORATORY PERSONNEL.

PROCEDURE
It is often necessary to share information concerning specific animals or cages of animals with others. This requires a standard method to describe and identify the exact location of an animal or cage of animals within an animal room. The method described herein, is used by LAR to identify a wide variety of portable, stationary and built-in caging systems.

- Present the location information for all multi-cage shelf rack systems in a rack (R), side (S), row (R), cage (C) format.
- This method of cage identification supersedes any manufacturer applied designations, except as described below for carousel style caging systems.
- The application of visual signs, tags, markings, etc. is optional.

ROOM LEVEL DESIGNATIONS:
1. Number each cage/cage rack in the room beginning with number one, i.e., R1. The cage rack designation may also include an abbreviation of the brand name or manufacturer name, e.g., T=Thoren, Tec=Tecniplast and OM=OptiMice, of the cage rack.

RACK LEVEL DESIGNATIONS (HORIZONTAL SHELVES):
2. Identify each side or definable section of each cage rack.
   2.1 Designate the first side/section encountered as side A, i.e., SA, continue until all sides/sections have received a sequential letter designation. This designation may be omitted for single sided cage racks.
3. Number all rows, whether occupied or not, from top to bottom, i.e., R1 and so on.

4. Number each cage space in each row, whether occupied or not, from left to right, i.e., C1 and so on.

Carousel Rack Level Designations (vertical columns):

5. Carousel cage racks do not have definable sides/sections; therefore, a side/section designation is not required.

6. Use the manufacturer applied letter designations for each column, i.e., A-J.

7. Number each cage space in each column, whether occupied or not, from top to bottom, i.e., C1-C10.

Examples: Following are examples of location descriptions as they might appear in communications between Animal Care, Veterinary and Investigator staffs.

- R1 R4 C5 indicates a single sided rack in the first position on the perimeter of the room, forth row from the top and the fifth cage from the left.
- T3 SB R2 C1 indicates a double sided Thoren rack in the third position on the perimeter of the room, second row from the top and the first cage from the left.
- OM2 F 10 indicates an OptiMice rack in the second position on the perimeter of the room, column F, tenth cage from the top.

Author: Steven K. Cary  BS, LATg—Animal Care Specialist—LAR   Date: 9/16/2011
Reviewed by: Kenneth Hines  BS, LAT—Coordinator—LAR   Date: 9/19/2011

Revision #1 by: Name—Title—Department   Date:
Reviewed by: Name—Title—Department   Date:

Add additional lines for each subsequent Review/Revision
Persons Responsible
All LAR Users

Purpose
The Room Specific Instructions Sheet contains investigator contact information and room specific special instructions.

Safety Considerations
PRINCIPAL INVESTIGATORS (PI) ARE RESPONSIBLE FOR THE SAFETY AND TRAINING OF THEIR STAFF

Procedure
The concept of room specific instructions implies that a situation exists that is not already covered by routine animal husbandry practices and must be formally addressed in some way. Animal Care supervisors/managers must review and approve the addition of any room specific instruction prior to implementation. The Room Specific Instructions Sheet provides space to record room specific information in five categories.

- Contact
- Breeding
- Enrichment
- Special
- Carcass disposal

Place one Room Specific Instructions sheet per animal room, usually posted on the inside of the animal room entry door.

1. Communicate with each Principle Investigator (PI) to identify at least one investigator staff member to act as the Contact person. Include both telephone number(s) and electronic contact information. Establish a preferred hierarchy if more than one Contact Person is identified.

2. Communicate with the PI and/or the LAR Breeding Coordinator to establish the level of responsibility of each party in animal rooms containing breeding colonies.

3. Communicate with the PI to identify any room specific environmental enrichment needs.

4. Communicate with the PI to identify any other room specific special needs or instructions.

5. Communicate with the PI, Animal Care supervisors/managers and/or LAR veterinary staff to identify any room specific special instructions for animal carcass processing and disposal.
Appendix
- Room Specific Instructions Sheet

| Author: Steven K. Cary BS, LATg—Animal Care Specialist—LAR | Date: 12/19/2013 |
| Reviewed by: Denise Ostmeyer BS, CVT, LATg—Staff Trainer—LAR | Date: 8/13/2014 |

Revision #1 by: Name—Title—Department
Reviewed by: Name—Title—Department

Add additional lines for each subsequent Review/Revision
<table>
<thead>
<tr>
<th>Room Specific Instructions</th>
<th>Room__________</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact:</td>
<td>Breeding:</td>
</tr>
<tr>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>Enrichment:</td>
<td>Special:</td>
</tr>
<tr>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>Carcass Disposal:</td>
<td></td>
</tr>
</tbody>
</table>

Do not post additional signs for animal care technician instructions. To modify these instructions contact the animal care supervisor Sheryl.Carter@colostate.edu or 491-6571.
Persons Responsible
Investigator staff
LAR Veterinary and Technical Services staff
LAR Animal Care staff

Purpose
To describe the use of Isoflurane gas anesthesia in rodent species.

Safety Considerations
NOTE: THE PI IS RESPONSIBLE FOR THE SAFETY AND TRAINING OF THEIR LABORATORY PERSONNEL.

Procedure
Isoflurane gas anesthesia may be used for both recovery and non-recovery procedures in rodents. Humane euthanasia of rodents can be achieved by intentional overdose with Isoflurane gas while under anesthesia.

1. Prepare the gas anesthesia machine for use.
   1.1 Ensure an adequate supply of medical grade oxygen is attached to the oxygen regulator of the gas anesthesia machine.
   1.2 Ensure an adequate supply of liquid Isoflurane is in the anesthesia machine reservoir.
   1.3 Attach necessary anesthesia machine accessories, including, but not limited to, hoses and airbags to create one or more breathing circuits to deliver a breathable oxygen/anesthetic gas mixture to the animal and scavenge anesthetic gas laden exhalent away from the animal.
   1.4 Breathing circuits may include additional accessories, including, but not limited to, anesthesia induction chambers and nose cone masks.

2. Prepare the procedure work surface, e.g. table, counter top, biosafety cabinet, etc.
   2.1 Disinfect the work surface with a broad range disinfectant.
   2.2 Cover the work surface with towels, drapes, etc. to insulate the work surface to help maintain animal body temperature during anesthesia (optional for terminal procedures).
   2.3 Supplement the fabric insulation with an accessory heat source, e.g. heated water blanket, heating pad, microwavable heat pads, etc. (optional for terminal procedures).

3. Deliver anesthetic pre-medications, as determined by the PI, protocol and/or LAR Veterinary and Technical Services staff.

4. Place the animal in the induction chamber.
5. Adjust the anesthesia machine to introduce a 5% isoflurane/95% oxygen mixture into the induction chamber at a flow rate of ~5 liters per minute.

6. Observe the animal for the effects of the anesthetic gas.

7. Remove the animal from the induction chamber when the animal is at or near a surgical plane of anesthesia.

8. Position the animal on the procedure work surface.

9. Place a nose cone mask with a non-rebreathing circuit on the animal.

10. Adjust the isoflurane/oxygen mixture, as required, to maintain the animal at a surgical plane of anesthesia.

10.1 Reduce the isoflurane concentration to 1-2% if the animal is to be recovered post procedure at a flow rate of ~1 liter per minute.

10.2 Maintain the isoflurane concentration at 5% if the animal is to be euthanized during or immediately post procedure at a flow rate of ~5 liters per minute.

11. Place ophthalmic lubricant in the animal’s eyes.

12. Monitor the condition of the animal and the depth of anesthesia during planned recovery procedures. Adjust the isoflurane concentration as required to maintain a surgical plane of anesthesia.

13. Reduce the isoflurane concentration to 0% at the conclusion of the planned recovery procedure.

14. Continue to provide oxygen until the animal exhibits signs of consciousness. Turn off the oxygen supply and remove the nose cone mask from the animal.

15. Observe the animal periodically until sufficiently recovered to be returned to its cage.

16. Administer post-operative medications and supportive care as determined by the PI, protocol and/or LAR Veterinary and Technical Services.

17. Process any animal carcasses (see LAR SOP Disposition of Animal Carcasses).

| Author: Elisa French BS, LATg—Veterinary Technician—LAR | Date: 6/11/2010 |
| Reviewed by: Steven K. Cary BS, LATg—Animal Care Specialist—LAR | Date: 6/15/2010 |

Revision #1 by: Name—Title—Department

Reviewed by: Name—Title—Department

Date:

Add additional lines for each subsequent Review/Revision