

A 1 v1: Conventional Animal Facility Entry/Exit

Effective date: 3/31/2020

Approved by: Lon Kendall DVM, PhD; Director-LAR

Signature: 

Author: Michelle K. Adams, BS, LATg, CMAR; Quality Assurance Coordinator—LAR Date:2/17/2020
Reviewer: Jessica Ayers DVM, DACLAM; Associate Director—LAR Date:2/25/2020
Reviewer: Denise Ostmeier BS, CMAR, LATg; Assoc. Director Operations—LAR Date:2/25/2020
Reviewer: Ashley Creighton BS, LAT; Training Coordinator—LAR Date:2/25/2020
Reviewer: Janel Trumble LATg; Animal Care Supervisor-LAR Date:2/25/2020

Purpose:

To describe the requirements and procedures for entry and exit of animal facilities and rooms at Colorado State University (CSU) and to maintain all species in a clean and healthy environment 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 and to maintain biosafety and biosecurity as recommended by the Biosafety in Microbiological and Biomedical Laboratories (BMBL) manual.

Safety Considerations:

Allergens may be present throughout the facility. Animals are unpredictable and may present a physical hazard.

Procedure:

- Footwear must be closed toe.
 - Director's approval is required for persons under the age of 14 years to enter any LAR managed vivaria.
1. Plan work duties to follow LAR's established alphabetical room order based upon species and biosafety level (A through E, see Appendix). A clothing change and/or shower may be required to return to lower levels.
 2. Enter the facility or vivarium. If a keycard reader is present, do not allow persons unknown to you to follow you through the door. Those who do not have access require an escort during their visit.
 3. Read and heed all signs/white boards posted in animal facility areas.
 4. Proceed to the desired room (key card access may be required).
 - 4.1 Read and heed the Required PPE sign at the room entrance. Determine which PPE items must be acquired and donned prior to room entry and which items are available inside the room.
 5. Enter the room.
 - 5.1 Don required room specific PPE as indicated on room sign.
 6. Perform work related duties.
 7. To exit the room:
 - 7.1 Remove and store room specific PPE. Discard and replace, as needed.
 - 7.2 Remove and discard the disposable gloves.
 8. Proceed to the next scheduled duty or exit the facility, showering out from contaminated areas as required.
 9. Exit the vivarium and enter the appropriate locker room.
 10. Remove facility attire and footwear, if applicable. Place in the appropriate laundry containers or storage locations.
 - 10.1 Shower if required.
 11. Don "street" clothing and footwear.
 12. Wash hands before leaving the facility.

Appendices:

Room Order/Required PPE Sign
Facility chart

A 1 v1: Conventional Animal Facility Entry/Exit

Effective date: 3/31/2020

Pathology SPF cats

Room: Example

PPE to enter room:

None

Heed these requirements

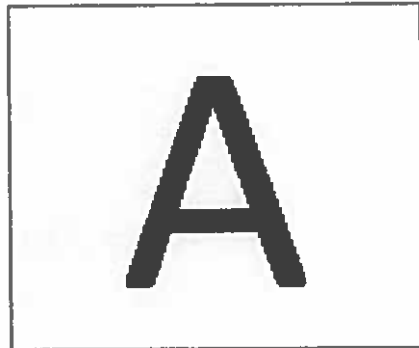


Additional PPE/ instructions to handle animals:

Gloves

Facility lab coat

Follow the room order



A

SPF Mice

B

SPF Other Rodents

C

Non-Rodents

D

ABSL2 or ABSL3



Quarantine/ Known Rodent Pathogen



Potential biohazard:

None

LAR Conventional Entry/Exit Chart

<u>Building</u>	<u>Main Entrance</u>	<u>Scrubs</u>	<u>Locker Room Location</u>	<u>Animal Room Location</u>	<u>Special Notes</u>
Painter Center	South side	Just inside Southeast entrance	Just north of entrances	Through door north of locker rooms	
Pathology	North or south entry	Not stocked, need to wear your own from Painter	None	Basement	See specific cat entry appendix
VTH	West side near silo	B116D	Women: B119 Men: B120	B114, B116, B117	
RIC	Through round tower lobby	D40 Hallway	Women: D47 Men: D46	Basement	
RBL	Through courtyard	AN140 Hallway	Women: A142 Men: A141	AN130 Hallway	No piggybacking! Everyone must scan their keycard.
ARBL	Central breezeway	Not stocked, need to bring your own	Women: EN119 Men: EN103	East wing	
Rampart	West door	Not stocked, need to bring your own	Off anteroom	South & East wings	
Biology	Any	Not stocked, need to wear your own from Painter	None	2nd & 3rd floors center	
A/Z	Any	Not stocked, need to wear your own from Painter	None	3rd floor of west wing	
PSRL Barn	South side	Facility-specific coveralls & boots required - stored & returned to TMI	TMI Room 109	PSRL Barn	See Q fever appendix regarding pregnant livestock. No re-entry through lobby of TMI unless boots are clean. Used LAR scrubs/socks must be returned to Painter for laundry.
EORC Barn	South main door	Facility-specific coveralls & boots required	None	West wing of horse barn	Coveralls & boots are in the LAR storage area cabinet. EORC laundry return to Painter for laundry

Q Fever Appendix



- Contracted by inhaling aerosols from birth products of infected sheep/ goats
- The Q fever status of our sheep/ goats is unknown
- Births/ abortions that occur on pasture are reported to and cleaned up by the barn crew
- Births/ abortions that occur in the barn –
 - Wear an N-95 and nitrile gloves until the area is cleaned and disinfected
 - Bag up birth products and contaminated bedding, tie closed, dispose in regular trash
 - Use a 1:32 bleach solution (1/2 cup bleach/1 gallon water) to disinfect any hard surfaces
- Notify LAR vet on-call of any births/ abortions

Pathology - Special Requirements for SPF Cats:

Obtain clean scrubs from Pathology Room 5.

Enter the P16 locker room (keycard required).

Remove street clothes, socks, and shoes (undergarments may be retained).

Be sure to retain your keycard.

You may don scrubs either before or after your extended hand washing.

Enter the shower and place scrubs (if not already donned) & keycard through the inner shower door.

Thoroughly wash hands and arms up to the scrub sleeve.

Upon exiting the shower, don scrubs (if needed), socks, mask, and headcover.

Do not forget your keycard!

Proceed to the common anteroom to don facility shoes.

Don gloves in the individual animal rooms.

Remove gloves when exiting each animal room.

Do not cross the chain barrier until ready to leave the SPF cat area.

Facility shoes must not cross the chain barrier.

Exiting SPF cats:

In common anteroom, remove facility shoes

Exit from the cat area by crossing to the other side of the chain or back through shower and discard disposable PPE in the trash

Re-enter the locker room to change into street attire.

Once you have changed, take your used scrubs, socks, and towel to the laundry bins in P5.

D101 Appendix

Animals may be temporarily transported to offsite laboratories by investigator staff for Institutional Animal Care and Use Committee (IACUC) approved experimental procedures. Research staff will follow these procedures.

Animal exit

- Prepare, in advance, by obtaining a clean sheet/pillowcase (available from the LAR Administrative Offices) and, if necessary, a transport cart.
- Enter the desired animal room (LAR SOP A 001)
- Cover the desired cage(s) with a clean sheet/pillowcase and remove it/them from the animal room.
- Exit the facility

Animals temporarily held off site for experimental procedures and with the intent to be returned to the Painter Center MUST be returned to the D101 Transitional Animal Holding Suite. They MUST NOT be returned to the main Painter Center vivarium.

Animal entry

- Cover the cage(s) with a sheet/pillowcase during the return to the Painter Center.
- Enter the D101 suite through the south door.
- Disinfect cages by wiping all exterior surfaces with pathogen specific disinfectant dampened paper towels.
- Return cages to their assigned D101 animal rooms.

Animal Carcass/Soiled Equipment Return. Carcasses of animals euthanized off site and any soiled equipment should be returned to the Painter Center (see LAR SOP #LGP 020 Animal Carcass/Soiled Equipment Return).

A 31 v2: Biosafety Cabinets and Change Stations

Effective date: 3/31/2020

Approved by: Lon Kendall DVM, PhD; Director-LAR

Signature: _____



Author: Denise Ostmeyer BS, CMAR, LATg; Assoc. Director Operations—LAR

Date: 12/13/2019

Reviewer: Jessica Ayers DVM, DAACLAM; Associate Director—LAR

Date: 12/17/2019

Reviewer: Sheryl Carter LAT; Facilities Manager—LAR

Date: 1/30/2020

Purpose:

Biosafety Cabinets (BSCs) and change stations enhance biosafety and biosecurity during the performance of animal husbandry and research activities in both conventional and ABSL-3 biocontainment barrier settings. They provide an environmentally controlled work space as a complement to Individually Ventilated Caging (IVC) systems. Change stations are primarily, but not exclusively, used in conventional settings and BSCs are primarily, but not exclusively, used in ABSL-3 biocontainment barrier settings.

Procedure:

1. Adjust any movable window sash to the manufacturer recommended working height.
2. Turn on, and allow the BSC/change station to run long enough to establish an air curtain. Follow manufacturer recommendations if available, or allow ten minutes prior to commencing work. BSCs in some ABSL-3 containment facilities are intentionally maintained on and running at all times as a component of normal air flow management and therefore do not need to be turned on.
3. Disinfect the work surface before use.
4. When working in Select Agent areas, or where infectious or recombinant material is present, cover the BSC work surface with absorbent liner soaked in a pathogen specific disinfectant.
5. Arrange any materials and supplies necessary for the desired procedure in and around the BSC/change station. Assure maximum air flow.
 - 5.1. Minimize the amount of materials and supplies placed in the BSC/change station at any one time.
 - 5.2. Keep all air flow grates and grids clear and free of materials, supplies and debris.
 - 5.3. Arrange materials and supplies in a clean to dirty directional flow.
6. Perform the desired procedure.
 - 6.1. Minimize disruptions to air flow patterns around the BSC/ change station by minimizing traffic around the cabinet, and using slow, deliberate movements in and out of the cabinet. This is particularly important when working in an ABSL-3 area.
7. When finished, remove any remaining materials and supplies.
8. Clean and disinfect the work surface after every use.
9. Thoroughly clean and disinfect the BSC/ change station periodically. This includes raising or removing the work surface to clean below, as well as all interior surfaces (walls, ceiling) of the cabinet. This should be done following cage changes, and as needed following other activities.
10. Replace pre-filters, if present, and/or clean any air intake screens at least monthly, or as needed. (See Guideline: Everything you ever wanted to know about filters).
11. Turn off the BSC/ change station when finished. BSCs in some ABSL-3 containment facilities are intentionally maintained on and running, at all times as a component of normal air flow management and therefore should not be turned off.
12. BSCs and change stations must be certified at least annually, or whenever they are serviced or moved from one building to another. This activity is coordinated by the facilities manager.

Revisions:

7/9/2014

B1 v3: Animal Welfare and Room Checks

Effective date: 1/5/2021

Approved by: Lon Kendall DVM, PhD; Director-LAR

Signature: 

Author: Michelle K. Adams BS, LATg, CMAR; Quality Assurance Coordinator-LAR Date: 10/29/2020

Reviewer: Denise Ostmeyer BS, CMAR, LATg; Assoc. Director Operations-LAR Date: 11/5/2020

Reviewer: Ashley Creighton BS, LAT; Training Coordinator-LAR Date: 11/5/2020

Reviewer: Janel Trumble LATg; Animal Care Supervisor-LAR Date: 11/5/2020

Reviewer: Kate Bruner PSM, CMAR, LATg, CLABP; Animal Care Supervisor-LAR Date: 11/20/2020

Purpose:

Animals are observed daily to verify general health and wellbeing and to assure intact, functional housing and access to adequate supplies of food and water.

Procedure:

1. Observe all animals daily for signs of illness, injury or death (see Rodent Behavioral Scoring Appendix).
 - 1.1 Provide immediate assistance to address and/or correct any situation placing animals in danger (see Urgent Care Appendix).
 - 1.2 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, initials and a brief description of the problem.
 - 1.3 Report sick, injured or dead animals in the LAR Animal Medical Records (AMR) System
 - 1.3.1 Read and follow the Room Specific Instructions (RSI) for PI-specific instructions regarding morbidities and mortalities.
 - 1.3.2 Utilize the AMR web application, to enter the case.
 - Enter the cage card number, which will auto populate many of the fields.
 - Verify the auto populated fields for accuracy, and enter missing information including protocol number, room number, location, PI, contact person, strain, DOB, receipt date, behavior score, USDA ID if applicable, problem.
 - Click "Create a New Case".
 - 1.3.3 Some animals housed in ABSL3 areas must be immediately reported to the research staff as noted on the RSIs. If the research staff cannot be reached, then follow steps in 1.3.2.
 - 1.3.4 Animals which are Rodent Behavioral Score C or D must be also reported immediately, in person or by telephone, to the LAR On Call Veterinarian at (970) 566-3414.
 - 1.4 Make note of any separations or mortalities on cage cards
 - 1.4.1 Adjust the cage card population count to reflect the number of remaining animals and write a dated, initialed note at the bottom of the card.
 - 1.4.2 If no animals remain in the cage, date and write "terminated" on the card and submit as required
2. Perform and document any required medical treatments or veterinary directives as prescribed on Clinical Observations Cards (red cards) or Animal Treatment Sheets (red sheets).
 - 2.1 If there are no veterinary exam notations on the Clinical Observation Card during the daily check after the initial report, re-report the AMR case as described in 1.3.2
3. Report and document any remarkable events in Breeder cages (see Breeder Cage Appendix).

B1 v3: Animal Welfare and Room Checks

Effective date: 1/5/2021

4. Process animal carcasses as needed (see LAR SOP B 035—Disposition of Animal Carcasses).
5. Assess food and water levels in every cage. Anticipate weekends and holidays and assure adequate supplies to reach the next regularly scheduled workday. Change/fill water bottles and/or add food, as needed.
6. Spot clean or change any animal environments that are overly soiled or wet.
7. Verify that basic facility functions, e.g. air handling, lighting, etc., and any mechanized caging systems are operational. Report any malfunctions to LAR Facility Manager.
8. Complete the Animal Husbandry & Room Monitoring Sheet.
9. Remove any dirty caging or supplies left by researchers, as needed.

Appendixes:

Urgent Care Required

Breeder Cage

Rodent Behavioral Scoring

Clinical Observation Card

Revisions:

12/15/2009, 12/29/2015

Urgent Care Appendix

Separate animals found **fighting** or with obvious/suspected fight wounds into new cages.

- Place a Clinical Observation Card (red card) on each separated cage with an injured animal, and report in the AMR system.
- Place a temporary cage card on each newly created cage, and submit a cage card request

Free **trapped appendages** and observe animal for a few minutes. If there is any sign of lameness, injury, or excessive grooming of the site then complete AMR.

Identify and remove, repair or replace **damaged or unsafe cages**, cage parts or equipment

- Move large animals to a clean, safe kennel or stall if needed until repairs are completed.
- Remove damaged equipment from circulation for assessment, inventory adjustment and disposal

Ulcerative Dermatitis – trim hind nails upon finding, place Clinical Observation Card, and submit AMR

Change **flooded cages**:

- Change the cage, providing appropriate enrichment and a new water bottle (see LAR SOP—Species Specific Husbandry)
- Attempt to diagnose/ correct the reason that the flood occurred.
- if the animals are dry or damp but otherwise appear to be unaffected, Behavior Score A or B (see Appendix—Rodent Behavioral Scoring), replace the dry cage back onto the rack. Reporting is not necessary.
- If the animals are wet, cold, or otherwise appear to be adversely affected, Behavior Score C or D:
 - Gently dry the animal hair coats, as much as possible.
 - 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.
 - Place a Clinical Observation Card on the cage, report it in the AMR system, and contact Vet On Call
 - Leave the cage on the heating pad overnight, leave a note in the Redbook about the cage and where it belongs on the rack.
 - Replace the cage onto the rack the following day.

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 gait 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 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

Clinical Observation Card

Investigator _____ CIC# _____
Date Reported _____ Tech _____
Vet Exam Date _____ Vet _____
Responsible for Treatment: AC V TS PI
Project# _____

Action Plan _____

Date	Initial	Date	Initial	Date	Initial

Re-check Date Date _____

B 34 v3: Carbon Dioxide (CO₂) Euthanasia of Rodents

Effective date: 5/28/2020

Approved by: Lon Kendall DVM, PhD; Director-LAR

Signature: 

Author: Ashley Creighton BS, LAT; Training Coordinator—LAR

Date: 3/25/2020

Reviewer: Jessica Ayers DVM, DACLAM; Associate Director—LAR

Date: 3/30/2020

Reviewer: Michelle K. Adams BS, LATg, CMAR; Quality Assurance Coordinator—LAR

Date: 3/30/2020

Reviewer: Denise Ostmeyer BS, CMAR, LATg; Assoc. Director Operations—LAR

Date: 3/31/2020

Purpose:

Rodents are routinely euthanized with CO₂ following the AVMA Guidelines for the Euthanasia of Animals.

Safety Considerations:

Pressurized cylinders must be stored and secured upright to prevent falling or being knocked over. Keep cylinders closed when not in use.

High concentrations of CO₂ can cause rapid suffocation, avoid inhalation of CO₂ gas. If inhaled and/or breathing becomes difficult exit room to fresh air.

Procedure:

1. Obtain a euthanasia chamber, preferably the animal's home cage.
 - 1.1 Do not overcrowd.
 - 1.2 Do not combine multiple species or incompatible animals.
2. Bring the rodents to one of the LAR provided euthanasia stations (see appendix – LAR Euthanasia Stations) or another appropriate CO₂ source. Only medical grade, compressed CO₂ may be used. Other sources (such as dry ice) are prohibited.
3. Refer to Appendixes: Directions for CO₂ Euthanasia of Rodents and LAR Large CO₂ Euthanasia Chamber and/or directions posted at the euthanasia station for directions and flow rates.
4. Confirm death by one of the following methods.
 - Perform a secondary physical method of euthanasia such as cervical dislocation, bilateral thoracotomy, perfusion, exsanguination, decapitation, etc.
 - Leave rodents in the CO₂ filled euthanasia chamber for a minimum of 5 minutes after visually confirming that all animals have stopped breathing.
 - Rodents <10 days old are resistant to death via CO₂ and should be euthanized via decapitation or cervical dislocation, with or without exposure to CO₂.
5. Process animal carcasses as required (see LAR SOP B 35 Carcass Disposal).
6. Transport soiled caging and equipment to the appropriate dirty equipment storage location.

Appendixes:

LAR Euthanasia Stations

Directions for CO₂ Euthanasia of Rodents

LAR Large CO₂ Euthanasia Chamber

Revisions:

7/12/2010, 5/20/2014

LAR CO2 Euthanasia Stations		
CO2 Setup Location		Special Notes (if applicable)
Main Campus	Painter Necropsy	CO2 tank setup
	Painter C113	CO2 tank setup
	Painter D101	For LAR staff use only. Please refer to LAR Large CO2 Euthanasia Chamber instructions
	Painter D102	CO2 tank setup
	Pathology Necropsy	CO2 tank setup
	Physiology 121	CO2 tank setup
Foothills Campus	ARBL E129	CO2 tank setup
	Pod 3 - RBL B151	Facility CO2 handle on ceiling
	Pod 3 - RBL B191	Facility CO2 handle on ceiling
	Level 2 - RBL A131b	Lid, flowmeter, facility CO2 handle on wet sink
	Phase 3 - C136	Lid, flowmeter, hose in cabinet next to BSC. Facility CO2 handle on ceiling across from BSC
	BRB 123a	Facility CO2 on counter
	BRB 110e	Facility CO2 on counter
	RIC D57	Facility CO2 on wall
RIC D58	Facility CO2 on wall	

Directions for CO₂ Euthanasia of Rodents-2020

1. Secure a euthanasia chamber, ideally using the home cage. Increasing animal density and mixing of different animals from separate cages in the euthanasia chamber should be avoided to decrease pre-euthanasia anxiety.
2. The volume of the euthanasia chamber (in liters) should be calculated and then divided by 2 (3.33-1.43) in order to determine the appropriate CO₂ flow rate 50% (30-70%) based on the 2020 AVMA Guidelines newly recommended displacement rate. If your current flowmeter does not measure high enough for the cage, you may either set it on the highest measured flow rate, or you may move animals into a clean smaller cage to use a lower calculated flow rate-see below.

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

#1 Mouse Thoren cage: 5.8L, flow rate of 3.0 L/min (1.7-4)

#9 Mouse Thoren cage: 6.5L, flow rate of 3.5 L/min (1.9-4.5)

OptiMice cages: 6.2L, flow rate of 3.0 L/min (1.8-4.3)

#2 Rat cage: 10.4L, flow rate of 5.0 L/min (3.1-7.3)

Tecniplast SealSafe (blue & green lines): 7.6L, flow rate of 4.0 L/min (2.3-5.3)

Deep Rat Cage (e.g. Ancare R20): 26.2L, flow rate of 13.5 L/min (7.8-18.3)

Tecniplast Rat/Hamster cage: 19.8L, flow rate of 10.0 L/min (5.9-13.8)

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. If there is a second gauge on your setup (B), then it should then be turned on as well (this one is usually left on each time so may not have to be adjusted). After both valves have been turned on, the flow meter can be adjusted to the appropriate flow rate (liters/min) in order to gradually introduce 100% CO₂ to result in the replacement of 30-70% of the cage volume per minute. This flow rate has been shown to produce a rapid loss of consciousness without apparent pain.

4. Following the induction of unconsciousness (this will take approximately 1-3 minutes), the CO₂ flow rate can be increased to accelerate the process. Following apparent clinical death of the animal (no visible respirations), gas flow should be maintained for at least one minute.

5. Following death the main tank valve should be turned off, and excess gas allowed to flow out of the tubing. Once gas has stopped flowing, turn the flowmeter valve off.

6. Cervical dislocation or bilateral thoracotomy should be performed to assure the animal will not regain consciousness.

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.

References:

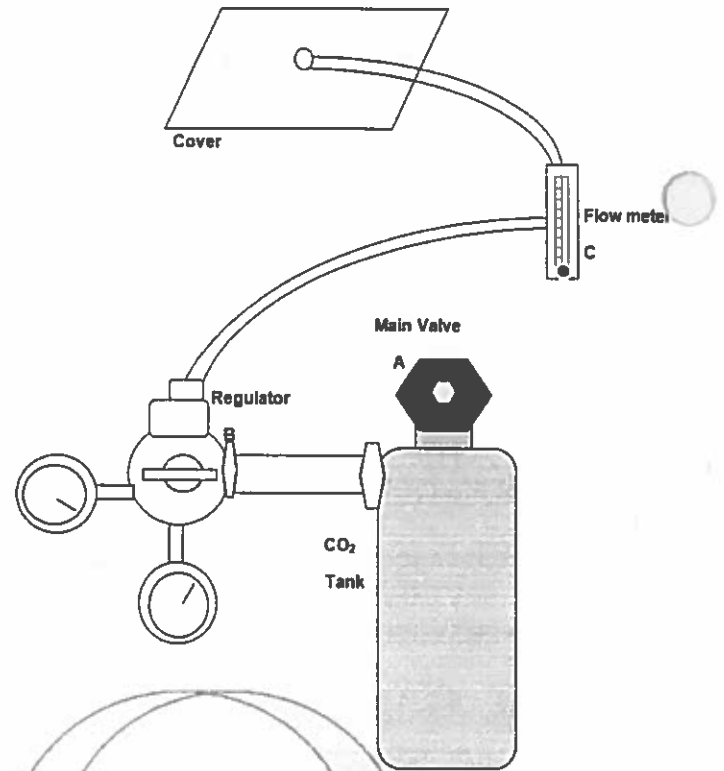
AVMA Guidelines on Euthanasia, January 2020.

[Revised 1/15/2020 to update flow rates based on newest AVMA recommendations.]

[Revised to add new cage type/volume 2/6/2018]

Proper Maintenance of CO₂ Equipment

1. Before turning the tank on, make sure the flowmeter (C) is turned off (all the way to the right). This eliminates the risk of blasting your animals with a high flow of CO₂.
2. Turn the main valve (A) only $\frac{1}{4}$ to $\frac{1}{2}$ turn to the left to open the tank. Even if very little CO₂ is left in the tank, this is all that is needed to let the gas flow.



Turn the main valve (A) and the flowmeter (C) **LEFTY LOOSEY** to open them.

3. The regulator valve (B) is turned the opposite direction. Turn the regulator valve **RIGHT** to start the flow of CO₂. Again, this only needs to be turned $\frac{1}{4}$ to $\frac{1}{2}$ turn. Some regulator valves may already be correctly set and no adjustments are necessary.

Turn the regulator (B) **RIGHT** to open it.

4. When you are finished, please do not leave pressure in the system, this increases wear and tear on the equipment and leads to repair/ replacement costs. The proper way to turn everything off is:
 1. Turn the main valve (A) all the way to the right.
 2. Wait for all of the gas to bleed out of the system. Increase the flowmeter (C) to speed this up.
 3. Once the gas stops flowing, and the pressure gauge on the tank reads 0, then turn the flowmeter (C) all the way to the right, and the regulator (B) $\frac{1}{4}$ to $\frac{1}{2}$ turn to the left.

Turn the main valve (A) and the flowmeter (C) **RIGHTY TIGHTY** to close them.

Turn the regulator (B) **LEFT** to close it.


Contact the LAR Training Coordinator to schedule a CO₂ demonstration.

LAR Large CO₂ Euthanasia Chamber:

1. Place cages in large container. Ensure that no cages are directly under the hose when the lid is placed on the chamber.
2. Chamber capacity:
 - #9 mouse cages – 10 total (stacked 2 high)
 - #1 mouse cages – 15 total (stacked 3 high)
3. Turn on the main valve on the CO₂ tank (turn left to open). CO₂ should begin flowing into the chamber. The regulator valve should be set between 15-20 L/min.
4. Monitor mice for apparent death (no visible respirations), approximately 8-10 minutes. Leave mice in CO₂ chamber for an additional amount of time to ensure euthanasia, approximately 3-5 minutes.
5. Turn the main valve on the CO₂ tank off (turn right to close). The regulator valve does not need to be turned off. Gas will continue to flow until the hose is emptied.
6. Mice not needed for necropsy purposes, and with ear tags removed, can all be placed in the same bag and placed in the freezer in D101 suite to be taken to Foothills at a later date.
7. There are 2 chambers that can now be used. While one chamber is in use the second can be setup with cages. Once the first chamber is complete the lid can be transferred to the second chamber- this can be done without turning the /CO₂ flow off while moving it between bins.
8. Please fill out the provided chart and time how long the CO₂ tank is on per bin to allow us to determine how long the tank lasts.

B35 v4: Disposition of Animal Carcasses

Effective date: 5/28/2020

Approved by: Lon Kendall DVM, PhD; Director-LAR Signature: 
Author: Janel Trumble CVT, LATg-Animal Care Supervisor-LAR Date: 2/7/2020
Reviewer: Ashley Creighton BS, LAT; Training Coordinator—LAR Date: 4/13/2020
Reviewer: Denise Ostmeyer BS, CMAR, LATg; Assoc. Director Operations—LAR Date: 4/13/2020
Reviewer: Michelle K. Adams BS, LATg, CMAR; Quality Assurance Coordinator—LAR Date: 4/13/2020
Reviewer: Jessica Ayers DVM, DACLAM; Associate Director—LAR Date: 4/13/2020

Purpose:

To store and dispose of animal carcasses in order to assure collection of samples, as required, and to assure both biosafety and biosecurity in the disposal of the remains.

Procedure:

1. Check the Room Specific Instruction (RSI) sheet for investigator specific instructions concerning the handling of animal carcasses and where they should be stored.
2. Place the carcass(es) in a plastic ziplock-type bag for small species, trash bags for larger species.
3. Seal and label bag(s) using permanent marker. Include at least the following required information:
 - 3.1. Cage card number
 - 3.2. Principle Investigator
 - 3.3. Date
 - 3.4. Indicate whether to “save” or “trash”
 - 3.5. Room Number
4. Place the bagged carcass(es) in short term cold storage (refrigerator/cooler) or freezer as directed by RSI. Separate bags marked “save” from bags marked “trash”, pending necropsy or disposal.
5. Submit an LAR Work Order Form when a cold storage location becomes full, to request that material handlers remove and dispose of the carcasses.
6. Special handling of animals maintained at ABSL-3
 - 6.1. Pre-treat the interiors of one Ziploc bag and one autoclavable bag with a pathogen specific disinfectant unless directed otherwise by the investigator.
 - 6.2. Place the carcass(es) in a Ziploc bag and then seal the bag.
 - 6.3. Place the bagged carcass(es) in an autoclavable bag and seal with autoclave indicator tape.
 - 6.4. Label the bag using a permanent marker and autoclave indicator tape as indicated in 3.1-3.5 above.

Also include:

 - 6.4.1 Your first initial and full last name
 - 6.4.2 Pathogen, if applicable
 - 6.5. Refrigerate or freeze, per investigator request, bags marked “save”.
 - 6.6. Periodically consolidate “trash” and other disposable, processed carcasses and tissues into larger autoclavable bags then autoclave out of the ABSL-3 containment barrier.
 - 6.7. Maintain autoclaved carcasses and tissues in cold storage (frozen) until they are disposed of in step 5 above.
 - 6.8. Select Agent carcasses should be stored as indicated on the RSI. Carcasses are disposed of by IDRC staff.

Revisions:

5/15/2009, 2/21/2012, 4/3/2014

B 044 v2: Room Red Book and Room Specific Instructions

Effective date: 12/16/2019

Approved by: Lon Kendall DVM, PhD; Director-LAR

Signature: _____

Author: Denise Ostmeyer BS, CMAR, LATg; Assoc. Director Operations—LAR

Date: 11/13/2019

Reviewer: Janel Trumble LATg; Animal Care Supervisor-LAR

Date: 11/14/2019

Reviewer: Jessica Ayers DVM, DACLAM; Associate Director—LAR

Date: 11/14/2019

Reviewer: Michelle K. Adams BS, LATg, CMAR; Animal Care Supervisor—LAR

Date: 11/14/2019

Purpose:

The Room Red Book provides a central location for LAR animal room documentation, and communication between various users of the room. The Room Specific Instructions provide a single document for communicating all specific needs of the room to the LAR Animal Care staff.

Procedure:

1. The following documents are kept in Room Red Books.
 - 1.1. Communication Log
 - 1.1.1. Use the log as an open channel of communication between Research staff, LAR Animal Care, Veterinary and Technical Services, and Supervisors.
 - 1.1.2. Maintain the log on a single laminated page.
 - 1.1.3. Make entries using the provided wet erase marker.
 - 1.1.4. LAR Animal Care staff are required to check the communication log every day.
 - 1.1.4.1. Identify and address any concerns or needs.
 - 1.1.4.2. Identify morbidity and mortality information entered by research staff and submit in the Animal Medical Records system (see LAR SOP AHGP 33 Animal Medical Records).
 - 1.1.5. Wipe the log clean at least weekly, when filled, or as issues have been resolved.
 - 1.1.6. Information that needs to be maintained for a longer period of time should be added to the Room Specific Instructions.
 - 1.2. LAR Contact List
 - 1.3. Rodent Behavioral Scoring Sheet (see LAR SOP AHGP 31 Animal Welfare Checks)
 - 1.4. Medical Abbreviations
 - 1.5. Cage Card Request Forms
 - 1.6. LAR ABSL-2 Animal Housing Information Form(s), as needed
 - 1.7. IDRC SOP: Escaped Mouse/ Hamster/ Rat (Animal) in Containment, as needed in BSL-3 locations
2. Room Specific Instructions (RSIs)
 - 2.1. Animal Care Supervisors create RSIs for every animal room. If updates are needed, contact the Animal Care Supervisor.
 - 2.2. RSIs are posted on animal room doors.
 - 2.3. Avoid posting other types of notes or instructions for Animal Care Staff.

Revisions:

8/13/2014

B 45 v2: Animal Identification**Effective date: 5/28/2020**

Approved by: Lon Kendall DVM, PhD; Director-LAR

Signature: 

Author: Ashley Creighton BS, LAT; Training Coordinator—LAR

Date: 2/6/2020

Reviewer: Michelle K. Adams BS, LATg, CMAR; Quality Assurance Coordinator—LAR

Date: 2/7/2020

Reviewer: Jessica Ayers DVM, DACLAM; Associate Director—LAR

Date: 3/31/2020

Reviewer: Denise Ostmeyer BS, CMAR, LATg; Assoc. Director Operations—LAR

Date: 3/31/2020

Purpose:

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

Procedure:

1. Identify all animals with a cage card.
 - 1.1. Cage cards are generated using the animal management software system for individual animals, or for cages/rooms of animals.
 - 1.2. Required cage card information includes:
 - 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)
 - Species
 - 1.3. Additional information that is commonly included:
 - Vendor (source)
 - Date received
 - Strain
 - Gender
 - Date of birth
 - 1.4. Cage cards that are generated for cages of animals must include the cage population number and gender. Update this information any time it changes.
2. Document animals found dead or separated from the cage by updating the total number in the cage, and making a note with the date, the number of individuals involved, any available ID number(s).
3. Identify animals that are awaiting a cage card with temporary cage cards. Common reasons that temporary cage cards are needed include:
 - Cage separations
 - Weaning of breeding colony progeny
 - Delivery of unexpected animals
- 3.1. Enter all requested information and place the Temporary Cage Card(s) on the appropriate cage(s).
- 3.2. Submit a Cage Card Request Form either on the LAR website or using a paper form.
- 3.3. Place the corresponding Permanent Cage Card(s) in front of the Temporary Cage Card and staple the cards together as soon as possible.
4. Dogs and cats must be identified with a USDA number. This is typically tattooed in their ear, as well as written on their cage card.

B 45 v2: Animal Identification

Effective date: 5/28/2020

5. Investigators may apply additional forms of identification such as ear tags or notches, tattoos, and microchips. When present, LAR staff should provide this information to investigators in the Animal Medical Record system, or other relevant communication.

Revisions:

4/10/2014



**LABORATORY
ANIMAL RESOURCES
COLORADO STATE UNIVERSITY**

STANDARD OPERATING PROCEDURES

LAR SOP # B 046

Version: 2

Category: Animal Husbandry—General Procedures

Title: Animal Cage/Rack Identification System

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

Signature: _____

Effective Date: **12/20/2011**
Revision #1 Date: **10/20/2019**

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

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

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 and working from left to right around the perimeter of the room, i.e., R1, R2, etc. An abbreviation of the brand/manufacturer name of the cage rack may be substituted for the R rack designation (see Appendix).

Rack Level Designations (horizontal shelves):

2. Identify each side or definable section of each cage rack.
 - 2.1 Mobile cage racks—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.

2.2 Double sided stationary cage racks—designate the left side as rack 1 (T1) and the two definable sections, working from left to right as sides A and B. i.e., T1 SA and T1 SB. Likewise, designate the right side as rack 2 (T2), again with two definable sections and again working left to right as sides A and B, i.e., T2 SA and T2 SB.

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.

Appendix

- A=Allentown
- OT=OptiMice
- T=Thoren or
- TP=Tecniplast

- o **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, side B, second row from the top and the first cage from the left.
 - OT2 F 10 indicates an OptiMice rack in the second position on the perimeter of the room, column F, tenth cage from the top.

Revision #1

- A. Clarify the cage numbering sequence.
- B. Define sides and sections of multiple cage rack styles.

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: Steven K. Cary BS, LATg—Animal Care Specialist—LAR	Date: 9/3/2019
Reviewed by: Jessica Ayers DVM, DACLAM—Associate Director—LAR	Date: 10/29/2019
Reviewed by: Denise Ostmeyer BS, CMAR, LATg—Assoc. Dir. of Operations—LAR	Date: 10/25/2019

Add additional lines for each subsequent Review/Revision