

**University of Virginia**  
**Center for Comparative Medicine (CCM)**

**Animal User Manual**

Version 2.2

**UVA Animal Ordering,  
Barrier Training,  
Animal Husbandry  
and Veterinary Care**



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CCM Information WEBSITE: <http://www.healthsystem.virginia.edu/internet/ccm>

## WELCOME AND INTRODUCTION

Welcome to UVa's biomedical research program. You are a contributor to on-going biomedical research enterprise that is leading to valuable treatments for a variety of disease.

The use of animals for research is a privilege bestowed under a public trust that is closely regulated by the federal government. Since you are reading this manual you are listed under an animal use protocol approved by the Animal Care and Use Committee (ACUC) and you have met the other requirements to be listed as an animal handler at UVa. This manual is designed to instruct you in how to obtain animals at the University, how they are housed and cared for, whom to contact if you have special animal husbandry or veterinary medical needs and many other facts in how to obtain materials and services to accomplish your approved research.

Most of the animals at UVA are rodents, and many of the husbandry practices used to maintain them are geared toward keeping them disease-free. To prevent the introduction of disease into our mouse barriers you should consider that anything you carry into the animal room be it yourself, your mice or any inanimate object are each capable of **INTRODUCING RODENT DISEASES**. Once a new disease is introduced into a mouse room it will spread at a rate that is dependent on susceptibility of the mice, the means by which it spreads, its inherent virulence characteristics and the mouse handling practices of people using the room. **ANY NEWLY INTRODUCED DISEASE WILL SPREAD UNTIL IT IS DETECTED; IT IS LIKE INTRODUCING FIRE INTO A DRY FOREST**. Once a new disease is acquired by naive mice in a barrier room it will continue to spread and perpetuate itself until drastic actions are taken to eradicate it. Experimental results will be significantly altered by the introduction of disease and the result is a waste of animal lives, your research time and money. **YOU ARE ON THE FRONT LINE OF PREVENTING THIS FROM HAPPENING TO YOUR RESEARCH AND OTHER RESEARCHERS AT UVA**.

The practices used to eradicate rodent diseases are very expensive and time-consuming. **WE MUST DO EVERYTHING WE CAN TO KEEP OUR MICE FREE OF OPPORTUNISTIC DISEASES**.

With your adherence to ACUC, IBC and CCM policies and practices outlined in this training manual, you will contribute to keeping research animals healthy, research results sound and reproducible, personnel and animal safe and secure as well as contributing to the success of the entire UVA research community.

If you have questions regarding this manual, see the vivarium supervisor for assistance or contact a CCM Veterinarian for guidance.

Sincerely,

Sanford Feldman, DVM, PhD, ACLAM Diplomat  
Professor and Director, Center for Comparative Medicine  
Attending Veterinarian  
Vice Chair, IACUC

## **FREQUENTLY ASKED QUESTIONS**

### **1. HOW DO I GET PERMISSION TO USE ANIMALS FOR RESEARCH OR TEACHING AT UVA?**

BEFORE ANYONE CAN USE ANIMALS AT UVA THEY MUST OBTAIN APPROVAL FROM THE ANIMAL CARE AND USE COMMITTEE (ACUC). CCM TRACKS ALL ANIMAL USE, including breeding, FOR THE ACUC TO MAKE CERTAIN ONLY APPROVED PERSONNEL USE THE NUMBER AND SPECIES OF ANIMALS THEY ARE PERMITTED AND DO NOT EXCEED ACUC APPROVED LIMITS. FOR MORE INFORMATION CONTACT THE ACUC OFFICE AT 4-0405.

### **2. HOW DO I ORDER ANIMALS FOR RESEARCH AT UVA?**

ALL ANIMALS MUST BE ORDERED THROUGH THE CCM WEBSITE AT <https://vprgsecure.web.virginia.edu/ccm/index.cfm>

- a. ONLY PERSONNEL LISTED ON APPROVED ANIMAL PROTOCOLS CAN ACCESS THIS SYSTEM, CONTACT 4-2324 OR 4-5058 FOR YOUR ID AND PASSWORD. FIRST TIME PIs MUST PROVIDE PTAO ACCOUNT INFORMATION, CALL 4-9984.
- b. TO IMPORT ANIMALS FROM ANOTHER INSTITUTION SUBMIT REQUEST FORM AT <http://www.healthsystem.virginia.edu/internet/ccm/docs/TORECEIVEANIMALS.pdf> AND FAX TO 4-0354.
- c. TO RECEIVE ANIMALS FROM ANOTHER LABORATORY AND/OR HAVE THEM MOVED BETWEEN LOCATIONS AT UVA SUBMIT REQUEST FORM <http://www.healthsystem.virginia.edu/internet/ccm/docs/ProtocolorDeliveryFORM.doc> BY FAX TO 4-0354, YOU MUST ALSO REQUEST THE MOVE FROM DR. FELDMAN BY E-MAIL [SHF2B@VIRGINIA.EDU](mailto:SHF2B@VIRGINIA.EDU).

### **3. WHAT IS A RODENT BARRIER FACILITY (VIVARIUM)?**

A RODENT BARRIER FACILITY IS DESIGNED AND USES PRACTICES AND PROCEDURES THAT IMPOSE BARRIERS TO RODENT DISEASE TRANSMISSION. PRACTICES SUCH AS GOWNING AND ASEPTIC CAGE HANDLING ARE SIGNIFICANT TO KEEPING A VIVARIUM FREE OF RODENT DISEASE.

### **4. WHY DO WE USE RODENT BARRIER FACILITIES?**

DISEASES IN RODENTS DRAMATICALLY ALTER EXPERIMENTAL RESULTS (IMMUNOLOGIC, PHYSIOLOGIC, REPRODUCTIVE AND BEHAVIORAL STUDIES). BARRIER PRACTICES PREVENT INTRODUCTION OF DISEASE INTO AND AMONGST OUR GENETICALLY ENGINEERED MICE (GEMS). LAB ANIMAL RESEARCH RODENTS INCLUDE: MICE, RATS, HAMSTERS AND GERBILS. ). FOR THE PURPOSES OF THIS DOCUMENT THE WORD MICE MAY BE GENERICLY INTERCHANGEABLE WITH RODENTS.

### **5. WHERE DO RODENT DISEASES COME FROM?**

THE GREATEST DISEASE RISK TO OUR GEMS IS FROM INFECTED RODENTS. INFECTED RODENTS CAN BE FROM THE WILD, IMPORTED WITH MICE FROM OTHER INSTITUTIONS, OR TRANSMITTED ON THE SURFACE OF OBJECTS, LIKE PEOPLE'S HANDS. IF YOU HAVE PET RODENTS AT HOME OR YOU FEED RODENTS AS FOOD TO YOUR SNAKES OR OTHER

PETS DO NOT ENTER OUR BARRIER FACILITIES. CONTACT THE CCM DIRECTOR TO DISCUSS ALTERNATIVES TO ENTERING A VIVARIUM IF YOU OWN RODENTS AS PETS.

## **6. WHO DO I CONTACT IF MY MICE ARE SICK?**

CCM HAS A STAFF OF THREE VETERINARIANS AND FIVE VETERINARY TECHNICIANS AS WELL AS A SOPHISTICATED DIAGNOSTIC LABORATORY. CONTACT DR. MATHEW KESSLER, ASSOCIATE DIRECTOR CCM, AT 4-2090 OR GINA WIMER, LVT, SENIOR VETERINARY TECHNICIAN, AT 4-5406 IF YOU FEEL YOUR MICE NEED VETERINARY ATTENTION. THE ANIMAL CARE STAFF LOOK AT ALL THE ANIMALS AT LEAST ONE TIME EACH DAY, 365/7, INCLUDING HOLIDAYS. THERE IS ALWAYS A VETERINARIAN ON-CALL FOR AFTER CORE VETERINARY WORK HOURS (M-F 7:00 – 5:00 P.M.) EMERGENCIES.

## **7. HOW DO I MOVE MY MICE TO OUR LABORATORY AFTER CCM CORE TRANSPORTATION HOURS (M-F 7:00 – 3:30 P.M.)?**

RODENT CAGES MUST BE ENCLOSED IN A PAPER OR PLASTIC BAG WHEN MOVED THROUGH PUBLIC SPACES SO THAT THE PUBLIC CANNOT SEE THEM. YOU SHOULD ONLY USE SERVICE ELEVATORS (NOT PUBLIC ONES) WHEN TRANSPORTING ANIMALS. YOU MUST ALSO PROTECT THE CAGES FROM INCLEMENT WEATHER OR TEMPERATURE VARIATIONS. DO NOT RETURN THE MICE TO A BARRIER ROOM AFTER THE MICE HAVE BEEN REMOVED FROM THE FACILITY, EACH VIVARIA HAS A RETURN AREA OR QUARANTINE ROOM FOR MICE THAT TRAFFIC OUT AND NEED TO RETURN.

**TREAT OUR GENETICALLY ENGINEERED MICE LIKE  
THE EXTREMELY VALUABLE RESOURCE THAT THEY ARE!**

### **VIVARIUM SPECIFIC INFORMATION**

**The following information will be of assistance to your orientation to the particular facility (ies) where your animals are housed.**

Your animals are housed in VIVARIUM NAME: \_\_\_\_\_

VIVARIUM SUPERVISOR'S NAME: \_\_\_\_\_

OFFICE PHONE NUMBER \_\_\_\_\_

AFTER HOURS PHONE OPERATOR FOR VETERINARIAN ON-CALL

E-MAIL ADDRESS \_\_\_\_\_

VIVARIUM ASSISTANT SUPERVISOR'S NAME: \_\_\_\_\_

OFFICE PHONE NUMBER \_\_\_\_\_

E-MAIL ADDRESS \_\_\_\_\_

VIVARIUM ROOM (OR AREA) FOR DIRTY CAGING RETURN: \_\_\_\_\_

LOCATION OF CARCASS REFRIGERATOR OR FREEZER: \_\_\_\_\_

LOCATION OF QUARANTINE HOUSING: \_\_\_\_\_

LOCATION OF ABSL2 HOUSING: \_\_\_\_\_

LOCATION OF SUPPLIES: \_\_\_\_\_

CAGING \_\_\_\_\_

FEED \_\_\_\_\_

STERILE WATER \_\_\_\_\_

BEDDING \_\_\_\_\_

BODY BAGS \_\_\_\_\_

PPE – GLOVES, GOWNS, SHOE COVERS, ETC. \_\_\_\_\_

LOCATION OF CO2 CHAMBER(S): \_\_\_\_\_

## VIVARIUM ACCESS CONTROL

1. You must be listed as an animal handler on an ACUC approved protocol to request access to the vivarium. Access to some vivaria is by cardkey and by key control at others.
2. You must complete barrier orientation training with the vivarium supervisor or his/her designee before you can request vivarium access.
3. To obtain access you must submit the attached vivarium-specific access form to the vivarium supervisor. The request takes 48 hours for cardkey access and slightly longer for key requests. Vivarium access form can be located on the CCM website.
4. You are not allowed to lend your cardkey or room key to another individual that has not met criteria 1 through 3 in this section. If you grant vivarium access to a non-authorized person, your vivarium access privileges are at risk of being revoked.

## VIVARIUM PRACTICES

1. Failure to follow the policies of the Animal Care and Use Committee or vivarium procedures of the Center for Comparative Medicine can result in denial of access to the animal facility. Willful disregard for animal welfare will be reported to the UVA Office of Animal Welfare and Animal Care and Use Committee. Information about the Animal Care and Use Committee and policies can be found at the following address: <http://www.virginia.edu/vpr/iacuc/>.
2. No food, beverages or gum are allowed in the vivaria at any time. Break rooms are available in the Vivarium for consumption of food and beverage.
3. The vivarium keys and card keys that provide you access to the facility and animal rooms are NOT to be given to any other individual. Anyone found distributing access codes or card keys to another individual might have their access revoked.
4. If you have had contact with rodents in a non-research setting (pet store, petting zoo, visiting the home of a rodent pet owner or feeding rodents to a pet rat snake) **you are not permitted entrance to a vivarium for 48 hours** without further review by the facility supervisor and CCM Director.

## ANIMALS ORDERS FROM COMMERCIAL VENDORS

1. All commercial research laboratory animals must be ordered through the CCM website at <https://vprgsecure.web.virginia.edu/ccm/index.cfm> . For instructions on how to use this website please contact the CCM office at 4-2741 or 4-2324.
2. Animal ordering deadlines by approved vendor are listed in the table below. The CCM staff strictly adheres to vendor deadlines.

<b>Vendor</b>	<b>Deadline</b>	<b>Delivery Day</b>
ABI	Wednesday at 11:00	Tuesday
Archer Farms	Wednesday at 11:00	Tuesday
BURLESON	Wednesday at 11:00	Tuesday
Carolina Biological Supply	Wednesday at 11:00	Tuesday
CBT FARMS	Tuesday at 8:00	Tuesday
Charles River	Wednesday at 11:00	Tuesday
CONNECTICUT VALLEY	Wednesday at 11:00	Tuesday
Covance Research Products (dogs)	Wednesday at 11:00	<b>Wednesday</b>
Cytogen Research & Dev.	Wednesday at 11:00	Tuesday
GENETIC MODELS, INC.	Wednesday at 11:00	Tuesday
Glades Herp, Inc.	Wednesday at 11:00	Tuesday
Harlan	Tuesday at 8:00	<b>Sunday</b>
Jackson	Thursday at 12:00	Thursday
Liberty Research, Inc.	Wednesday at 12:00	Tuesday
Marshall Biosources	Wednesday at 11:00	Tuesday
Moulton Chinchilla Ranch	Tuesday at 12:00	Tuesday
Mouse Models of Human Cancer Consortium	Wednesday at 11:00	Tuesday
Mutant Mouse Resource Ctr	Wednesday at 12:00	Tuesday
Myrtle's Rabbitry	Wednesday at 11:00	Tuesday
NASCO (ARISTOTLE CORP)	Wednesday at 11:00	Tuesday
National Cancer Institute	Tuesday at 12:00	Tuesday
National Institute for Aging	Tuesday at 12:00	Monday
Pet Forum	Wednesday at 11:00	Tuesday
Rat Resource & Research Ctr (RRRC)	Wednesday at 11:00	Tuesday
S & S Farms	Wednesday at 11:00	Tuesday
SASCO	Wednesday at 11:00	Tuesday
SHOTWELL, KAREN	Wednesday at 12:00	Tuesday
SINCLAIR RESEARCH CENTER	Wednesday at 11:00	<b>Thursday</b>
Slonaker Farms	Wednesday at 11:00	Tuesday
SPAFAS, Inc	Wednesday at 11:00	Tuesday
TACONIC	Wednesday at 11:00	Tuesday
Three Springs Scientific	Wednesday at 11:00	Tuesday
XENOPUS 1	Wednesday at 11:00	Tuesday
XENOPUS EXPRESS	Wednesday at 11:00	Tuesday

3. The CCM system compiles commercial animal orders by vendor, generates a list of incoming orders with associated notations specific to each vivarium, prints all cage labels based on the information you input to the system and debits animal orders

against ACUC protocol allowed limits (within a protocol year). If you have special requests on how the animals should be housed, place that information in the note section of your animal order. Your notes will appear on the list of incoming animals that the vivarium staff reads. Animals will be group housed on corn-cob bedding unless requested otherwise in the notes section.

4. Incoming mice are typically placed in the room you request in your order, typically where other mice under the same principal investigator are located. If you do not have an existing colony please consult with the vivarium supervisor regarding what room you should request for housing before placing your mouse order. If you are aware that your designated animal housing room is near capacity, please see your vivarium supervisor in order for you to designate an alternate housing space in your weekly order.
5. There is no formal notification of arrival of animals ordered from commercial vendors. You should assume animal orders would arrive within the week after you ordered them. If they are back-ordered, or if your order does not match what the vendor ships to the vivarium, you will be contacted by the animal ordering administrative support person.
6. If you are having difficulty placing an order contact the CCM office at 4-2741 or 4-9984.

There is an ACUC Acclimation Policy

((<http://www.virginia.edu/vpr/iacuc/docs/Acclimation.pdf>) requiring that animals be allowed specific periods of time after before being used in research. Please review this requirement before beginning experimentation on animals after they arrive.

## ASEPTIC TECHNIQUES FOR RODENT CAGE CHANGING AND OTHER BARRIER ROOM PRACTICES

1. When entering the barrier mouse rooms or working with mice in procedure rooms always put on the following disposable personal protective equipment (PPE): disposable gown, hair bonnet, surgical mask, shoe covers and gloves. Make certain you cover your wrist by pulling the glove over the gown sleeve.
2. Some of our mouse barrier rooms contain mice with abnormal immune systems (e.g. athymic nude, Rag 1-KO, Rag-2 KO, PFP-KO, SCID, SCID-NOD, Bg/Nu/XID, etc.). These mice with abnormal immune systems cannot fight off even a simple infection. Flora and fauna normally carried by our immune competent mice often make immune deficient mice ill, sometimes with dire consequences. Therefore, it is best to avoid housing immune competent mice with the immune deficient mice, if needed consult one of the veterinarians. It is best practice to handle immune deficient animals before handling immune competent mice in areas with limited space such as quarantine.
3. Inside the vivaria our best practices include the liberal use of high-level disinfectants (e.g. SporKlenz) on the outside of cages, surfaces and materials that may come into contact with the mice PARTICULARLY YOUR GLOVED HANDS. Liberally use high-level disinfectants (e.g. SporKlenz) on the outside of surfaces of materials that you are bringing into the mouse room. Make certain that anything you bring into the mouse room is free of any infectious material being either sterile or capable of being sprayed with SporKlenz. SporKlenz needs time to disinfect so allow at least five minutes when spray sanitizing objects.
4. Open mouse cages ONLY inside an animal transfer station (ATS) or a biosafety cabinet (BSC). Aseptic techniques are methods used to prevent contamination of the mice and everything else in the mouse barrier room. Aseptic techniques are laboratory practices that maintain the integrity of your research and of the state of the research animals. To reduce the risk of possible spread of undetected pathogenic microorganisms to mice it is essential that you follow guidance regarding following aseptic cage handling techniques. The animal transfer station (ATS) blower must be turned on prior to and during work in the cage change station. The sash must remain down for the HEPA air to flow properly.
5. When mice are taken to laboratories for manipulation, placed in open metabolic or behavior monitoring caging, moved to common use imaging areas or removed from the vivarium for any reason WE DO NOT ALLOW THEM BACK INTO THE BARRIER ROOMS. We place them in “quarantine” space where they do not put the barrier mouse rooms at risk.
6. Animals in the vivarium must always be assigned to an approved animal protocol and provided a cage card with other pertinent and required information. The animal care staff is responsible for ensuring that the information listed on the cage card will

remain with the animal(s) for the duration of their usage. If an animal is permanently removed from a cage at any time (e.g. males for fighting), the cage card information must be conveyed to the new cage. According to ACUC policy

([http://www.virginia.edu/vpr/iacuc/docs/Policy\\_on\\_Cage\\_Card\\_Information01-15-08.pdf](http://www.virginia.edu/vpr/iacuc/docs/Policy_on_Cage_Card_Information01-15-08.pdf)) cage cards must contain the following information: PI name, protocol number, CCM (billing) code, species, strain, sex, origin and weight or age.

Remember, the laboratory is responsible for making sure appropriate cage labels are placed on cages when mice are weaned. The vivarium supervisor can supply these labels.

7. Place only one cage of mice inside an ATS or a biosafety cabinet (BSC). Open only one occupied cage in the ATS at a time so that the identity of the mice is only associated with the card on the cage, and groups of mice are not mixed up regarding the cage labels.
8. **CHANGE YOUR GLOVES IF THEY HAVE HOLES OR RIPS.** Always wear gloves when working with mice whether in an animal room or procedure room. Liberally and frequently spray your gloves with Sporklenz.
9. Use Sporklenz in a spray bottle and a sterile disposable towel to decontaminate all surfaces of the hood work area both before and after using the ATC or BSC.
10. Spray all items you introduce into the ATS, including your gloves, and repeat spraying anytime you introduce an object into the ATS. Spray all surfaces of the cage except the white filter paper microisolator top and cage card with the spray disinfectant. Take care to spray the bottom of the cage where your gloved hand touched.
11. Decontaminate the ATS/BSC work surface (bench top) between each row of caging.
12. Cover cages with a filter top when outside the ATS and never place a cage on the floor inside an animal housing room or procedure room.
13. When placing a ventilated cage with automatic watering back in position on a rack, make certain it is pushed into the locked position (you will likely hear the lock click in position and see a red dot on some caging models disappear) or the mice cannot get to the sipper tube.
14. The number of animals in any cage must not exceed the number recommended by The Guide for the Care and Use of Laboratory Animals. A standard mouse Allentown JAG-75 or Thoren cage holds five adult mice, older Allentown caging that does not say JAG-75 only holds four adult mice per cage. See the ACUC policy on acceptable housing of rodents  
<http://www.virginia.edu/vpr/iacuc/docs/BreedingWeaningRodents.pdf>..

NIH Guide  
Table of Cage  
Capacity by  
species:

<b>ANIMAL</b>	<b>WEIGHT, grams</b>	<b>NUMBER OF ANIMALS PER CAGE</b>
Mice	<10 grams	12
	Up to 15 grams	9
	Up to 25 grams	6
	>25 grams	5
Rats	<100 grams	4
	Up to 200 grams	4
	Up to 300 grams	3
	Up to 400 grams	2

15. Clean cage components are most often available in the animal housing room. Ensure that caging components remain sterile by closing the container after use before removing the container from the ATS or BSC. Do not leave paper bags or cloth bags open in the room. Never place cages of animals or caging components directly on the floor in any animal room or procedure room. Sterile water is available in carboys in some animal rooms, and sterile feed is in labeled containers. If supplies are low (usually on a weekend) contact the vivarium supervisor or weekend supervisor for assistance.
16. Non-biohazardous soiled caging should be returned to the soiled side of cagewash and not left in the animal housing room. Soiled cages can be stacked, but the top cage should be covered to diminish airborne odors and allergens.
17. In animal rooms make certain to place trash in the appropriate receptacle:
  - a. Syringes, with or without needles/pipettes in red “Sharps” container.
  - b. Carcasses must be wrapped in black plastic bags and delivered to a carcass refrigerator.
  - c. General trash can be placed in labeled trash containers (labeled trash) and the lid tightly closed after use.
18. Animals should not be left in the ATS or BSC overnight. Rodents in caging must be returned to the ventilated rack as soon as possible following handling.
19. Make certain the ATS or BSC fluorescent light is turned off when you are done so the mice have night in their diurnal light cycle.
20. Make certain animal housing and barrier entrance doors are closed and secured immediately after entry or exit.

## RODENT DIETS

Standard laboratory animal diets are purchased from Harlan Teklad and they are either steam sterilized or purchased gamma irradiated for sterility. If you require special diets for your research, you are responsible for purchasing special diets. The use of specialty diets must be noted in your animal use protocol. Place a card on each animal's cage that is to receive a special diet containing the following information: special diet type, starting date for diet, and ending date for diet, so that the animal care staff know that a special diet is being administered. For CCM help with placing special diet purchases, contact 4-2052. **No diet can be fed past a six month (or as applicable, possibly three months for high fat diets) original mill date, or if the diet is moldy or compromised in any way.**

## DRINKING WATER

Drinking water for laboratory animals is always bacteria-free. The source of water is building tap water that is filtered at 0.2 microns in Jordan Basement, Gilmer Hall, Jordan Annex G211 and G14D or OMS. Building or local reverse osmosis water that is UV irradiated and/or 0.2 micron filtered is used in Aurbach, MR5, Snyder, LiSA, MR4 Annex and MR6. In other spaces tap water is steam sterilized before placing in water bottles (MR4, some areas of Aurbach and Jordan Annex). Water can be acidified to a pH of 2.5-3 upon request if you are having trouble with *Pseudomonas aeruginosa*, however you should consider the effect of the chlorine burden in the animal's diet on your research results. Consult with a veterinarian if you have questions. Special treatments in water such as trimethoprim-sulfamethoxazole for mice after irradiation and bone marrow reconstitution is supplied by the vivarium and the cost passed on to the laboratory. Doxycycline water for expression of genes under tetracycline promoters must be supplied by the laboratory. Remember doxycycline is sensitive to light so bottles should be amber or wrapped with sterile foil to protect the antibiotic from light.

## AUTOMATIC DRINKING WATER AND LIXIT TRAINING

Many of our facilities incorporate automatic drinking water systems as a continuously available source of high quality bacteria-free water. At the time of weaning mice or rats that are housed in a room with automatic watering must be training to use the automatic water (referred to as Lixit Training). Since this is often done by the research staff, we ask that you put a green Lixit Training quarter-card on the cage to designate weanling rodents undergoing Lixit Training. Any mice or rats separated from a cage during the lixit training process must be labeled with a Lixit Training Card with the information that was on previous card. Lixit training is performed as follows:

Make sure that the box has feed and a full water bottle. Get a "Lixit Training" card as shown below and place it in front of your cage card.

The 1<sup>st</sup> start date on the card is usually the date you wean the animal (or the incoming date from a vendor, another vivarium or copied from a previous card). The animal will have a water bottle kept on the cage for two weeks past that 1<sup>st</sup> start date.

The 2<sup>nd</sup> start date is when the water bottle is removed. The caretaker in the room will remove the bottle daily for seven days, tap the lixit on the rack so there is a droplet of water on it for the animal to find, and also watch the animals closely for signs of dehydration. Signs of dehydration are ruffled fur, hunched appearance, not eating so less feces and urine in the cage, etc. If this is seen the bottle put back on for two weeks and the process is repeated. Some of the mice and rats will not use the lixit and we will try to train again (but not usually the case).

<p>LIXIT TRAINING</p> <p>1<sup>ST</sup> START DATE _____</p> <p>2<sup>ND</sup> START DATE _____</p>
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### **BEDDING**

CCM purchases two types of bedding. The most commonly used is crushed corncob and less frequently used is hard wood chip. The advantage of crushed cob is its absorbency for urine; however, it does contain sugars and should not be used for fasting mice or fasting blood glucose determination. Hard wood chip has no sugars, poor absorbency but can be used to more easily to monitor urine production.

Specialized compressed bedding called Nestlets, a material mice used to build nests, is provided to all pregnant mice. Shepherd shacks are paper product that mice can hide in and build nests from view and higher light levels. Both bedding additives are used to redirect aggression of the dam away from her offspring. Nestlets are provided without charge. Shepherd shacks have a nominal charge for their use. Isotek pads are available for semi-paralyzed rodents to facilitate their wellbeing and are (with or without associated charges)?

### **SPECIALIZED CAGING**

CCM has metabolic cages for rodent research upon request. The use of metabolic caging must be described in the approved animal protocol. Please ask the appropriate vivarium supervisor to obtain caging needed. There are a limited number of metabolism cages and use may need to be scheduled with advance notice given to allow for cleaning and delivery. Once animals are transferred to the metabolic cages, transfer the animal cage card as well. Place an additional card on the metabolic cage that includes the date the animal was placed into the metabolic cage and the date that the animal will be removed from the cage.

### **EUTHANASIA**

Euthanasia techniques are specified in your animal protocol. The most common method is CO<sub>2</sub> inhalation, and less commonly anesthetic overdose. Each vivarium has at least one rodent euthanasia area where a chamber is located capable of being flooded with CO<sub>2</sub> from a compressed cylinder. Please contact the vivarium supervisor to have them demonstrate the use of the chamber and show you its location(s). A copy of the SOP on use of the CO<sub>2</sub> chamber is appended to this document. If you need to purchase anesthetic, contact the veterinary staff.

ACUC Policy dictates that method be used to confirm death after euthanasia or animal. For mice, performing either cervical dislocation or cutting into the chest are both suitable options. **IF YOU ARE PERFORMING CERVICAL DISLOCATION WITHOUT ANESTHESIA IT MUST BE APPROVED IN YOUR ACUC PROTOCOL.** Neonates require additional time, up to ten minutes, for CO2 euthanasia and require secondary confirmation of death by decapitation. Please review the ACUC policy on confirmation of death at ([http://www.virginia.edu/vpr/iacuc/docs/Euthanasia\\_Confirmation\\_of\\_Death\\_Carcass%20Disposal.pdf](http://www.virginia.edu/vpr/iacuc/docs/Euthanasia_Confirmation_of_Death_Carcass%20Disposal.pdf)).

Euthanasia request forms must be submitted at least 24 hours in advance of the procedure requested date (Appendix 2.). For easy identification and confirmation, it is best to mark the cage card with an “E” if the entire cage is to be euthanized. See Appendix 3 for a Euthanasia Request form.

### **TECHNICAL SUPPORT-TIME AND VET-TECH TIME**

1. Animal caregivers can perform a variety of services that are not covered by per diem, but are billed as services performed on a quarter hour basis. The following are common tech-time services that can be provided if the workload does not interfere with the animal caregivers’ husbandry duties:
  - a. Clip teeth for mice with malocclusion
  - b. Weaning or separating overcrowded cages of mice
  - c. Provision of soft food
  - d. Administration of medications and/or research injections
  - e. Euthanasia (see request form Appendix 3)
  - f. Tail biopsy and application of individual identification (eartag)
  
2. Veterinary technicians can provide technical assistance as well as medications such as anesthetic and analgesics. To obtain medications from the veterinary technicians, use the form in Appendix 8:UVA Center for Comparative Medicine Drug Request Form or at URL [http://www.healthsystem.virginia.edu/internet/ccm/forms\\_and\\_documents.cfm](http://www.healthsystem.virginia.edu/internet/ccm/forms_and_documents.cfm) . Veterinary technicians can be scheduled to perform any of the following services that are billed on the quarter hour:
  - a. Tail vein injections
  - b. Anesthesia and anesthetic monitoring
  - c. Surgical site preparation
  - d. Gavage
  - e. Blood collection
  - f. Parenteral administration of medications, analgesics or experimental substances

### **RODENT WORKSHOP AND RODENT SURGERY WORKSHOP**

The UVa Office of Animal Welfare provides training in rodent biotechnology and rodent anesthesia and aseptic surgery workshops at no cost to the participants. These workshops are popular and hold a limited number of participants on a first signed-up first trained basis. For information on when these workshops are available and how to enroll go to URL

<http://www.virginia.edu/vpr/animalwelfare/classes.html>

## **MOVEMENT OF ANIMALS OUT OF BARRIER ROOMS AND THE VIVARIUM**

1. Movement of animals between buildings during normal working hours is performed by CCM. To request transport by CCM personnel to another building, mark the cage to be moved and submit an animal delivery request from URL <http://www.healthsystem.virginia.edu/internet/ccm/docs/ProtocolColorDeliveryFORM.doc> *at least 24 hours prior to the procedure requested date and time.* If the animal is moving to another vivarium or being transferred between animal protocols **you must also send an e-mail to Dr. Sanford Feldman (shf2b@virginia.edu) requesting the move**, including all details in the protocol/delivery form, in order to secure veterinary health approval for the transfer between buildings. Dr. Feldman will promptly correspond by e-mail with the supervisors of both vivaria to ensure space is available. **The same delivery request form and e-mail to Dr. Feldman must be completed to transfer animals between animal protocols.**
2. When rodents are taken to laboratories for manipulation, placed in open metabolic or behavior monitoring cage, moved to common use imaging areas or removed from the vivarium for any reason, **WE DO NOT ALLOW THEM BACK INTO THE BARRIER ROOMS.**
  - Rodents returned to the vivarium are placed in “quarantine” housing space where they do not put other barrier mouse rooms at risk.
  - Animals can be released from this quarantine if needed when laboratory testing for opportunistic infections are negative. Serologic testing in quarantine of a sentinel co-housed with the animals to be surveyed for the three-week period will be billed to the research lab.
3. When removing a cage of mice from the vivarium, conceal the cage in a brown paper bag to obscure it from public view. Paper bags are available for covering cages for transportation outside the Vivarium in public corridors to a lab in the same building. Moving the cage within restricted lab corridors not open to the public do not require the cage to be covered during transport to the lab.
4. If you are moving the cage to a laboratory, take a route that does not use public corridors if possible, and use service elevators not passenger elevators. **REMEMBER KEEP THE CAGE SEALED IN A PAPER BAG AND AWAY FROM PUBLIC AREAS.**

5. If you are moving a mouse cage to a procedure room and returning it to an animal housing room, you must spray the outside of the cage at the door entrance to the animal housing room
6. Mice and rats may not stay outside of the animal facility for more than 24 hours without prior authorization (i.e. ACUC approval). This is considered satellite housing and is only permitted with strong scientific justification. USDA regulated species such as other rodents, rabbits, dogs, cats, ferrets, and swine are not permitted to be outside of the vivarium for more than 12 hours without satellite housing approval.
7. If after hours or weekend transportation of animals is required, research personnel may do so with the following caveats:
  - i. The animals are transported in a cage that is obscured from view by the public by placing it or covering it with an opaque material
  - ii. Animals are transported in a manner that keeps them out of inclement weather (too hot, too cold, raining, etc.).
  - iii. Animals are transported in a manner that uses service elevators (not public elevators) and research corridors (not public corridors) as much as possible.

## **EXPERIMENTS INVOLVING ANIMAL BIOSAFETY LEVEL 2 (ABSL2) ORGANISMS OR CHEMICAL HAZARDS**

1. ABSL2 and experiments involving biological hazards require IBC approval. Experiments involving chemical hazards are reviewed and approved by EHS personnel on the IACUC. There is a separate orientation with the vivarium supervisor for personnel performing these experiments. Know your responsibility for notification of the vivarium supervisor when working with ABSL2 and chemical hazard agents. Signage must be posted on the cage (specialized stickers) and on entrance door of areas where biohazards and chemical hazards are present. Investigators are responsible for contacting the vivarium supervisor and removing cage stickers once the hazardous period has passed. The vivarium supervisor will remove the door sign once notified.
2. Almost all vivaria have an animal room set aside to accommodate ABSL2 investigations and where chemical hazards are used. Each designated room contains a BSC, SHARPS container, and a contaminated materials container for disposal of PPE and other contaminated items with appropriate biohazard or chemical hazard cage labels.
3. Exposed ABSL2 caging is steam sterilized prior to soiled bedding being discarded. Soiled cages are to be left bagged and labeled with a Biohazard sticker/label in the ABSL-2 room. Chemical hazard cages are to be bagged and marked with a chemical hazard label. All labels must include ABSL2 or chemical hazard agent to reduce risk to vivarium personnel.
4. For further training and information regarding ABSL2 experiments or chemical hazards, contact the vivarium supervisor or veterinarian.

## SPECIAL CAGE CARD DESIGNATIONS

### Animal Surgery Card

Contact Person \_\_\_\_\_

Telephone or PIC# \_\_\_\_\_

Animal ID \_\_\_\_\_

Surgery date \_\_\_\_\_

Procedure(s) \_\_\_\_\_

Post-op analgesic(s) \_\_\_\_\_  
and/or monitoring

Observation/Tx	Date/Time	Initials

Antibiotic(s) \_\_\_\_\_

Suture/clip removal date \_\_\_\_\_

### Post Operative Animal Surgery Card

1. The ACUC has a policy on rodent surgery (see Appendix 4 or [http://www.virginia.edu/vpr/iacuc/docs/Policy\\_Rodent\\_Surgery\\_09-16-08.pdf](http://www.virginia.edu/vpr/iacuc/docs/Policy_Rodent_Surgery_09-16-08.pdf)). A portion of this policy requires that an Animal Surgery Cards (red) be placed by the researcher/technician on the cage to document the date of the surgery and post-surgical care of the animal.
2. **Animals are to be returned to housing only when they are alert and mobile. Animals are not to be left unattended by the researcher until the animal is able to hold itself upright.**
3. Rodent Surgery cards are available from your facility supervisor. Rodent animal housing rooms and procedure rooms usually have a supply of Animal Surgery cards.
4. The research staff must specifically examine each post-operative animal daily for signs of pain (i.e., hunched posture, anorexia, or aggression including self-mutilation), and if noted in the animal protocol to administer analgesics. The period of post-procedural monitoring is specified in the approved animal protocol.

5. If any unanticipated complication is noted, contact the investigator and veterinarian immediately to determine what is best for the animal and the experiment. An after hours research contact number is helpful to the vivarium supervisor.
6. If no staples / sutures are present, the card should be moved to the rear of the cage cardholder once the incision has healed (usually 7-10 days). If sutures or staples are present, they should be removed when the wound is healed (usually 7-10 days).
7. Animal Surgery Cards for animals found dead will be attached to a Mortality Card. Adult mortality is reported each work day Monday through Friday. Weekend mortalities are reported each Monday.

### Animal Treatment (“Sick”) Card

1. Animal care staff observes every animal, at least once each day every day of the year. If animals are sick, cages will be marked with a yellow Animal Treatment Card (ATC).

2. Once the cage is presented as having an ATC, a veterinarian or veterinary technician will:

- i. Review the health of the animal and make a clinical assessment (Diagnosis is often written in red ink)
- ii. Determine if / when veterinary follow-up is necessary
- iii. Prescribe required animal care or research treatment for a determined period of time
- iv. Recommend euthanasia
- v. Or consider the case as resolved; requiring no follow-up.

3. **DO NOT remove Animal Treatment Cards from the cage.** If the animal is receiving on-going treatment for conditions such as malocclusion, the caretaker will make regular documentation of teeth clipping on the card.

<b>Animal Treatment Card</b>	
Room/Cage: G323 2AR1C5	
P.I./Protocol #: Smith 2999	
Date:	
Problem: raw irritated skin	
Diagnosis: Lesion	
Initial Rx/Management: Silvadene	
<b>Follow-up/Comments</b> (use back, if needed, for more space)	

4. If the animal requires administration of regular medication as prescribed by the veterinary staff, a separate treatment sheet will be produced for documentation of medical care and billing purposes.
5. If / when the case is resolved, the ATC will be placed behind the cage card for future reference.

Record #: _____		<b>ANIMAL MORTALITY CARD</b>	
Date:	Bldg:	Rm:	Protocol:
PI #:		Sex: Male <input type="checkbox"/> Female <input type="checkbox"/>	
DOB:	Date of Arrival:	Species:	
Found Dead <input type="checkbox"/>	Euthanized <input type="checkbox"/>	# Dead:	
Animal ID and/or Rack Location:		Was animal on Tx? Yes No <input type="checkbox"/> <input type="checkbox"/>	
Age		<input type="checkbox"/> pup	<input type="checkbox"/> adult
Reported By:	Notified By:	Person Notified:	

6. If the animal is found dead, an Animal Mortality Report (white card) is completed and both the mortality card and ATC are returned to the vivarium supervisor's office for daily reporting to the researcher. If the researcher finds a dead animal, the ATC card needs to be returned to the caretaker or office with a Mortality card. Mortality card supplies are in storage containers in each animal housing room and procedure room. Researchers do not need to complete a mortality card for normal euthanasia of animals at the end of experimental use.

## VETERINARY CARE IN BARRIER ROOMS

### Sentinel Monitoring Program

1. Sentinel rodents are placed strategically in every vivarium room housing rodents and the cages are left uncovered. The purpose of the sentinels is to detect the presence of

opportunistic disease in the mouse barrier room. These sentinels are exposed to dirty bedding from up to 120 cages on each rack; they are located on the bottom of the mouse rack toward the side of the exhaust ventilation. Once every 90 days a sentinel is removed and submitted to the CCM diagnostic laboratory for microbial profiling.

2. Sentinel mice are tested every 90 days for antibodies to the following diseases: mouse hepatitis virus, house parvovirus types 1 and 2, minute virus of mice, epizootic diarrhea of infant mice, Theiler's meningoencephalitis virus and mouse norovirus. The pelage is checked for ectoparasites and the intestinal tract examined for nematode parasites.
  - a. Some additional testing is done for bacterial pathogens when indicated.
3. Once a year a sentinel is submitted from each rodent room. The mice are annually tested for mouse hepatitis virus, house parvovirus types 1 and 2, minute virus of mice, epizootic diarrhea of infant mice, Theiler's meningoencephalitis virus, mouse norovirus, ectromelia, K-virus, Mycoplasma pulmonis, mouse thymic agent, mouse cytomegalovirus, reovirus-3, lymphocytic choriomeningitis virus and polyomavirus.
4. Results of sentinel testing are available from the CCM veterinary staff.
5. Non-routine testing is conducted at veterinary discretion for atypical health observations or unexpected or increased mortalities/morbidity.

## **COMMON MOUSE AILMENTS**

### **DYSTOCIA**

Dystocia literally means difficulty giving birth. This is very common in transgenic mice and is an emergency condition. Mice typically deliver at night, so a pregnant dam found with one pup (live or cannibalized) in the cage is in dystocia. Other clinical signs include copious vaginal discharge, the female's flanks are very sunken and she is lumpy looking, finally if the female appears hunched/lethargic/moribund.

The condition is best treated immediately. A cesarean section (female is euthanized) has the greatest rate of success in producing a live litter, however the dam is euthanized during this procedure. CCM maintains Swiss Webster foster females on hand to raise the C-sectioned litter. There is a charge for the cesarean procedure and foster female, however, depending on the value of the litter this may be of no concern.

Medical management of dystocia is less successful and entails treatment with three drugs: calcium gluconate, mesoprostal and oxytocin. We have found the greatest success is with calcium gluconate. Medical treatment is recommended when the dam is valuable and effort is made for her to survive. Finally, if the dam and litter have no intrinsic research value, they should be humanely euthanized with carbon dioxide.

If you find a female in dystocia, please alert the vivarium supervisor immediately to report this to the veterinary staff, or page PIC 2165 and report the condition. Remember, time is of

the essence in saving unborn neonates! If you want a valuable dam's neonates delivered, you may mark the cage, "perform C-section" if dystocia occurs. Prior cage labeling, planning and notification to the veterinary and animal care staff is an important time saver.

## ULCERATIVE DERMATITIS

This skin condition of mice that have C57BL/6 in their background is very common and aggravated by the feeding of high fat diets. The condition appears with one or several full thickness defects of the skin leaving a raw ulcerated area. The condition is amenable to treatment with immune suppressive drugs that undoubtedly would interfere with research results particularly those that involve inflammation.

Breeding animals can be treated, but the condition recurs many times when treatment is discontinued. Animals with this condition that cannot be treated must be euthanized when the skin defect (or defects) reaches the size of a dime in total. It is not humane to allow these animals to live a long period of time with raw ulcerated wounds.

## FIGHTING OF MALE MICE

Young adult and juvenile male mice that are housed together often fight, sometimes to the death. This is particularly noteworthy with C57BL/6 and BALB/c males including transgenic mice with these genetic backgrounds. Often the males are purchased together at a young age and begin to fight shortly after they are introduced into an animal room where they can smell the pheromone of female mice. Adult males of these inbred strains that were not housed together when young ALWAYS fight.

Animal caregivers are instructed to separate male mice that fight. Often these mice have to be housed one mouse per cage, although the males can be used in breeding boxes. **If the animal care staff has separated male mice for fighting you must never house them together again.**

Male mice that have been severely wounded must be treated or euthanized. Severely wounded male mice likely will not make suitable research subjects, depending on the research being performed. Therefore, the following table lists the density that male mice on a C57BL/6 and BALB/c background will be housed at, which is dependent on age:

Age (in weeks)	Number of male mice per cage
4-6	4 – 6*
6-8	3
8-10	2
10-12	1

\* based on weight and cage dimension

## **RECTAL PROLAPSE**

This condition is most often seen in immune deficient mice that have contracted an infection with one of the nine mouse *Helicobacter* species. It is caused by hyperplasia of the colonic mucosal and is not truly a prolapse. The most common bacterial causes of rectal prolapse are *H. hepaticus*, *H. bilis* and *H. rodentium*. Many of the immune competent mice at UVa harbor one or more of these bacteria. If you place an immune deficient mouse in a cage with immune competent mice, the immune deficient mouse often develops this lesion within a month.

This condition cannot be effectively treated with antibiotics. To eradicate this bacterium from immune deficient mice it is necessary to re-derive the line. If you are interested in re-deriving a line to a *Helicobacter*-free state, please contact a member of the veterinary staff.

## **HEAD TILT**

Twisting of the head (torticollis) with one ear toward the cage floor, turning obsessively in a circle to the same side, rolling and spinning when held by the tail are all clinical indications of a middle ear infection (otitis media) in mice. We have catalogued over 25 different bacterial species that can cause this problem at UVa. If diagnosed early the condition is amenable to treatment. If allowed to progress, this condition often leads to the demise of the infected mice.

## **TUMOR – ENDOGENOUS AND EXOGENOUS**

The most common tumors seen in mice are lymphoproliferative in origin. Lymphosarcoma is the most common cancer mice develop, with the liver, spleen and kidney being most commonly involved. These organs look dramatically enlarged, pale and often the mice appear pale as the circulating cancer cells crowd out normal bone marrow production of red blood cells.

Human tumors implanted into immune deficient mice (xenograft) require approval by the Institutional Biosafety Committee (IBC) as well as the ACUC. The ACUC has a policy about growing tumors in mice and we provide it here as Appendix 4 and can be found at URL [http://www.virginia.edu/vpr/iacuc/docs/Policy\\_Rodent\\_Surgery\\_09-16-08.pdf](http://www.virginia.edu/vpr/iacuc/docs/Policy_Rodent_Surgery_09-16-08.pdf).

## **ABSCCESS, FURUNCULOSIS (hair follicle infection) PREPUTIAL GLAND ABSCCESS AND METRITIS**

These conditions are caused by several different bacteria, most commonly *Staphylococcus aureus*, *Staphylococcus xylosus* and *Pasteurella pneumotropica*. These can be seen in immune competent or deficient mice. Generally these are treated (except metritis – uterine abscess) with an appropriate antibiotic established from isolating the offending bacterium. Consult the veterinary staff to obtain a culture and sensitivity.

## APPENDIX 1: CCM CONTACT INFORMATION: ADMINISTRATION, VETERINARY STAFF, AND VIVARIA CONTACTS

<u>Center for Comparative Medicine</u>	<u>Phone/Pager Number &amp; Email</u>
<b>CCM Administration</b>	
Sanford H. Feldman, DVM, PhD, Director	924-5058 (PIC# 2165) <a href="mailto:shf2b@virginia.edu">shf2b@virginia.edu</a>
Matthew J. Kessler, DVM, Associate Director	924-2090 (PIC# 2233) <a href="mailto:mjk4b@virginia.edu">mjk4b@virginia.edu</a>
Penny Pittman, Animal import and export	924-2052 <a href="mailto:plp2y@virginia.edu">plp2y@virginia.edu</a>
Linda Johnson, Animal Ordering vendors	924-2741 <a href="mailto:ldc@virginia.edu">ldc@virginia.edu</a>
Micki Laman, Office Manager and fiscal technician	924-9984 <a href="mailto:msl7c@virginia.edu">msl7c@virginia.edu</a>
Timothy R. Reid, Web Manager, Access Control	924-2324 <a href="mailto:trr9r@cms.mail.virginia.edu">trr9r@cms.mail.virginia.edu</a>
<b>MR4 vivarium</b>	
Alice Kenney, Supervisor MR4 Vivarium	924-5293 <a href="mailto:ask5g@virginia.edu">ask5g@virginia.edu</a>
Laurie Edwards Assistant Supervisor	<a href="mailto:lrc8r@Virginia.EDU">lrc8r@Virginia.EDU</a>
<b>OMS vivarium</b>	
Audrey Martin, Supervisor OMS Vivarium	924-0151 <a href="mailto:abm8h@virginia.edu">abm8h@virginia.edu</a>
Shawn Anderson, Assistant Supervisor	<a href="mailto:sma7d@Virginia.EDU">sma7d@Virginia.EDU</a>
<b>MR5 vivarium</b>	
Karen Oehrli, MR5 Vivarium Supervisor	243-9388 <a href="mailto:karen.oehrli@crl.com">karen.oehrli@crl.com</a>
Vivian Hofler, LATg, Asst Supervisor, MR5 Vivarium	<a href="mailto:vivian.hofler@crl.com">vivian.hofler@crl.com</a>
<b>Jordan Annex vivarium</b>	
Shelly Verling, Supervisor Jordan Hall Vivarium	243-6695 <a href="mailto:smv3p@virginia.edu.com">smv3p@virginia.edu.com</a>
Ayoub Tambire, Assistant Supervisor	<a href="mailto:ast2a@Virginia.EDU">ast2a@Virginia.EDU</a>
<b>Jordan Basement vivarium</b>	
Kim Dean, Supervisor, Jordan Basement vivarium	982-6991 <a href="mailto:kdd2z@virginia.edu">kdd2z@virginia.edu</a>
<b>Gilmer vivarium</b>	
Amy D. O'Coin, Supervisor Gilmer Vivarium	924-0690 or 982-5410 <a href="mailto:ado3f@virginia.edu">ado3f@virginia.edu</a>
<b>Aurbach vivarium</b>	
Marty White, Supervisor Aurbach Bldg Vivarium	243-6606 <a href="mailto:marty.white@crl.com">marty.white@crl.com</a>
Joy Gunter, LAT, Asst Supervisor	<a href="mailto:joy.gunter@crl.com">joy.gunter@crl.com</a>
<b>Snyder and LiSA vivaria</b>	
James Weirich, LATG, TMG contract mgr, Supervisor	243-8038, <a href="mailto:jweirich@themccgroup.com">jweirich@themccgroup.com</a>
Merritt Newman, LAT, Assistant Supervisor	<a href="mailto:mnewman@themccgroup.com">mnewman@themccgroup.com</a>
<b>Emerging Technology Center-1</b>	
Linda Rumery, Animal Caretaker	924-8126, <a href="mailto:linda.rumery@crl.com">linda.rumery@crl.com</a>
<b>Veterinary Staff</b>	
Gina Wimer, LVT, LATG Supervisory Vet Technician	924-5406 (PIC# 1845) <a href="mailto:grw8m@virginia.edu">grw8m@virginia.edu</a>
Jeremy Gatesman, LVT, LATG Supervisory Vet Technician	924-0477 (PIC# 4145) <a href="mailto:jjg4w@virginia.edu">jjg4w@virginia.edu</a>
Linda McVay, Veterinary Technician	982-0953 (PIC# 3680) <a href="mailto:lm7s@virginia.edu">lm7s@virginia.edu</a>
Kimberly Hellems, LVT, Veterinary Technician	982-0953 <a href="mailto:kjh5d@virginia.edu">kjh5d@virginia.edu</a>
Regina Campbell, LVT, Veterinary Technician	982-0953 <a href="mailto:rc8sm@Virginia.EDU">rc8sm@Virginia.EDU</a>
Weekend Vivarium Supervisors – Jordan, MR4, OMS, Gilmer	
Abraham Ntenda, Saturday	243-2955 (PIC# 2005) <a href="mailto:amn3f@virginia.edu">amn3f@virginia.edu</a>
Milica Vukovic, Sunday	924-5293 (PIC# 2005)

<sup>1</sup> To page someone dial 500 or 982-3500, enter PIC#, then a call back telephone number followed by the # key.

<sup>2</sup> Area code = 434 unless otherwise specified

## APPENDIX 2: POLICY ON BREEDING AND WEANING OF RATS AND MICE

Federal regulations establish the density of animals permitted in rodent cages. Breeder rats and mice and their litters can occupy considerable cage space, produce large amounts of fecal and urinary material, and increase cage temperature, humidity, and ammonia levels, all of which can create unhealthy conditions. When litters of two different ages are present in the same box, the younger litter often receives insufficient milk. Finally, the presence of more than one male in a breeding cage often results in aggression between competing males and fetal resorption (Bruce effect).

The following standards provide allowable densities and age/sex combinations to be applied to mouse breeding cages. Special exemptions to these standards can be made that improve animal well being, such as to accommodate weanlings of low birth weight, slow growth rates, or insufficient mothering; a typical solution might be to cross-foster to other females in the cage. Requests for ongoing exceptions for particular strains should be submitted to the ACUC by revision of your animal use protocol, in the Main Procedure section under a heading "Request for Exception to the Breeding and Weaning Policy". Exceptions must be justified by providing published citations or PI data demonstrating postnatal failure-to-thrive of the strain in question. Potential reasons for exemption(s) include: 1) Experimental design (e.g. studying psychological imprinting of pups); 2) Need to extend suckling in strains that fail to thrive (requires supporting data); 3) Need to prevent cannibalism diminished by cross-fostering (requires data).

### BREEDING SCHEMES AND LITTER NUMBERS ALLOWED:

- No more than one adult male is allowed in a breeding cage.
- Female rodents must be at least 6 weeks old before they are placed in a breeding cage with a male who is greater than 10 weeks of age. The only exception to this is superovulation of 3-4 week old female mice for collection of zygotes or embryos.
- A cage is considered overcrowded if a new litter is born before the older litter from the same female is weaned, or if the older litter remains with the female beyond 23 days of age. Litters with  $\leq 4$  pups do not require weaning separation if the male is removed during pregnancy.
- Weaning beyond 23 days (25 days of age if CCM does it) is reserved for strains that have litters that routinely fail-to-thrive. In such cases, the male, or even better the pregnant female must be removed from the cage when either female is obviously pregnant (e.g. by 12 days gestational age). Note: **Late weaning of this sort requires a special exemption as defined below to avoid ACUC censure.**

Animal caretakers are responsible for recording birth dates for litters on cage cards, however research staff may record DOB as well.

**A. One male x one female:** Litters should be weaned by postnatal Day 23, or before birth of another litter, whichever is first. Animal care technicians will automatically wean each litter by postpartum Day 25, or whenever a new litter is born, if investigators have not already done so. **Investigators will be charged for this service.** If there is no prior arrangement with the CCM staff to wean for the PI, it will be considered a noncompliance issue by the ACUC.

**B. One male x two females:** If breeding is conducted in a 1:2 ratio, it is recommended that one of the two females be removed to a separate cage when observed to be pregnant\*. Alternatively, it is permissible to keep the male, two females, and the pups together provided that when the oldest litter reaches 12 days of age there **are no more than 12 pups in the cage (combined)**. This is based upon standards used at the Jackson Laboratory (Reeb, Whittaker et al, 2001, Laboratory Animals 35:5873). Weaning must still be accomplished by Day 23. The animal care technicians will wean

litters at Day 25 if not already done by the investigators, and **investigators will be charged for this service**. If there is no prior arrangement with the CCM staff to wean for the PI, it will be considered a noncompliance issue by the ACUC.

**C. One male x three or more females:** If more than two females are housed with a male for breeding, pregnant females (e. g. 12 days gestational age\*) must be separated into boxes containing no more than two pregnant females before any delivery, and the 1:2 policy above (“B.”) is then followed. If removal of obviously pregnant females in this breeding scheme is not done by the research staff, the animal care technicians will perform this function and **investigators will be charged for this service**. If there is no prior arrangement with the CCM staff to wean for the PI, it will be considered a noncompliance issue by the ACUC. **The accepted procedure for post Day 25 day weaning:** Occasionally, litters (or individuals in a litter) do not thrive or there may be other extenuating circumstances to justify delayed weaning. If you feel a litter (as defined in this policy) is not ready to be weaned, consult your vivarium supervisor. Each supervisor has the authority to provide a onetime exemption (one cage, or for concurrent cages of a new strain) delaying weaning up to 9 days in length for any specific litter (normally weaned on Day 23, extension to 32 days maximum). If a new litter is born to another female in the cage during the exemption period, it must be removed with that dam to another cage, if the original exempted litter cannot be weaned. Permission for ongoing weaning age exemptions for a particular breeding line must be obtained from the ACUC by submitting a protocol modification for that strain. **Note: during any delayed weaning situation, the male must be removed from the cage when either female is obviously pregnant (e.g. by 12 days of gestation) to avoid postpartum estrus breeding.**

\*If you are unsure how to determine if a female is pregnant, ask the vivarium supervisor for assistance. *Vivarium supervisors will maintain a log of weaning exemptions in their office for inspection by the ACUC. (A sample log is appended.)*

## RODENT WEANING EXEMPTION LOG FOR SUPERVISORS

Vivarium/Supervisor Name: \_\_\_\_\_

Date Exempted	Principal Investigator	Protocol Number	Species	Date of Birth	Date to Wean	Exemption	Vivarium Room	Supervisor's Initials

**Note:** A Supervisor can only grant a weaning extension of  $\leq 9$  days beyond the original 23-day weaning age (i.e.  $\leq 32$  days of age). Exemptions  $> 32$  days can only be given by order of a CCM veterinarian.

**APPENDIX 3: COMPARATIVE MEDICINE ANIMAL EUTHANASIA REQUEST**

Investigator's name: \_\_\_\_\_ Protocol No: \_\_\_\_\_

Special hazards to personnel: \_\_\_\_\_

Code on which animals are being charged: \_\_\_\_\_

Species: \_\_\_\_\_ Animal I.D. Number: \_\_\_\_\_

Total number of animals to be euthanized:

\_\_\_\_\_  
Date: \_\_\_\_\_

**Signature of Investigator or Person Requesting Animal Euthanasia**

\_\_\_\_\_  
Date: \_\_\_\_\_

**Signature of Comparative Medicine Employee Euthanizing Animals**

-----cut-----cut-----cut-----cut-----cut-----cut-----cut-----cut-----cut-----cut-----cut-----cut-----cut-----

**COMPARATIVE MEDICINE ANIMAL EUTHANASIA REQUEST**

Investigator's name: \_\_\_\_\_ Protocol No.: \_\_\_\_\_

Special hazards to personnel: \_\_\_\_\_

Code on which animals are being charged: \_\_\_\_\_

Species: \_\_\_\_\_ Animal I.D. Number: \_\_\_\_\_

Total number of animals to be euthanized:

\_\_\_\_\_  
Date: \_\_\_\_\_

**Signature of Investigator or Person Requesting Animal Euthanasia**

\_\_\_\_\_  
Date: \_\_\_\_\_

**Signature of Comparative Medicine Employee Euthanizing Animals**

## APPENDIX 4: POLICY ON RODENT SURGERY AND POSTOPERATIVE CARE

### PURPOSE:

The purpose of this document is to outline procedures for aseptic surgery and postoperative Care for rodents. The U.S. Public Health Service *Guide for the Care and Use of Laboratory Animals* states that survival surgery on rodents should be performed in facilities intended for that purpose, using aseptic procedures to prevent clinical infections. The Animal Care and Use Committee also believes that judicious use of anesthetic and analgesic agents to minimize pain associated with the experimental procedure is scientifically and ethically imperative. This document clarifies expectations for aseptic technique and appropriate pre- and postoperative care.

### NonSurvival

#### Surgery

1. All animals must be sufficiently anesthetized prior to surgery such that they are completely unconscious and show no reaction to pain eliciting procedures. For most rodents, a lack of response to toe pinching is indicative of surgical anesthesia. Refer to the Center for Comparative Medicine guidelines for anesthesia for appropriate anesthetic agents and dosages.  
<http://www.healthsystem.virginia.edu/internet/ccm/Anesth/aneshome.cfm>
3. All animals are euthanized before recovery from anesthesia.
4. All personnel handling the animals must wear a labcoat, scrubs, or gown, and gloves.
5. Instruments and work surfaces need not be sterile but must be clean.
6. The surgical site should be free of hair.
7. Expired anesthetic and analgesic drugs **may not** be used under any circumstances. Other expired drugs can be used but should be specifically labeled "Nonsurvival Procedures Only" for this use.

### Survival Surgery Aseptic

#### Technique

1. Surgery must be conducted on a clean, uncluttered lab bench or table surface. The surface should be covered with a clean drape.
2. Hair must be removed from the surgical site using clippers, hair plucking, or a depilatory agent. A complete sterile prep of the surgical site consists of 3 alternating wipes with a disinfectant scrub (e.g. povidone iodine or chlorhexidine) and alcohol (or a sole agent such as Hibiclens), followed by application of an antiseptic solution. Placing a sterile drape over portions of the body that are not aseptically prepared is recommended to avoid contamination of the surgical site and/or suture material.
3. All instruments must be sterilized, with the method of choice based on the physical characteristics of the material to be sterilized. Sterile Supply located in the Hospital will autoclave or gas sterilize instruments that are properly wrapped. The Center for Comparative Medicine can also steam sterilize upon request. Sterilization indicators (heat sensitive tape) should always be placed inside the wrapped pack to confirm that the materials reached the appropriate temperature. Write the sterilization date on the outside and use within 6 months; re-sterilize if beyond 6 months. Hot bead sterilizers are inexpensive and very convenient for bench top use, especially for performing surgery on multiple animals consecutively. Acceptable techniques for cold sterilization include soaking in 2% glutaraldehyde for 10 hours or 8% formaldehyde + 70% ethyl alcohol for 18 hours.

There are several commercially available germicidal agents that are safer to use, but attention must be paid to the shelf life of solutions once they are prepared. Gauze pads, intravascular catheters, suture material, etc. should also be sterile. Please note placing instruments in 70% alcohol alone is **not** acceptable. Supplies obtained from “MERCİ” that have been previously opened should be re-sterilized.

4. The surgeon must wash his/her hands with an antiseptic surgical scrub preparation and then aseptically put on gloves. If working alone, the surgeon should have the animal anesthetized and positioned and have at least the first layer of the wrapped instruments or cold pack opened before putting on sterile gloves, so that he/she can remain sterile. Opened sterile items should be set down on a sterile surface.
5. The surgeon must wear a head cover and face mask. A sterile gown is recommended, but a clean scrub top, lab coat, or non-sterile gown worn over street clothes is acceptable.
6. Ophthalmic ointment must be administered in both eyes of the anesthetized rodent to protect the corneas from drying and abrasion.

### **Multiple Animal Surgeries**

If performing surgeries on more than one animal consecutively, **START WITH A STERILE INSTRUMENT PACK**. The instruments can be placed in 70% ethyl alcohol in between animals and preferably the instrument tips re-sterilized in a glass bead sterilizer, assuming there has been no gross contamination of the instruments (such as touching an unsterile area or object, or heavily contaminated with blood and/or tissue). A new sterile pack should be used after five surgeries. The alcohol should be replaced when moderately contaminated with blood or other body fluids. Sterile gloves should be changed between surgeries if the surgeon touches nonsterile surfaces. The surgeon may wipe his/her gloves for 30 seconds with sterile gauze pads soaked in alcohol between animals that are already prepped by another individual. If the surgeon is also performing animal prep between surgeries, he/she must use new sterile gloves for each animal.

### **Wound Closure**

The abdominal or thoracic body wall should be closed with absorbable suture material in a simple interrupted pattern. The skin should be closed with staples, wound clips, surgical adhesive, or with a nonabsorbable suture material in a simple interrupted pattern. Do not use silk for skin closure. A subcuticular closure can be used in larger rodents. Nonabsorbable skin sutures or staples should be removed when adequate healing is apparent, typically 7 to 14 days after surgery. If the survival time postsurgery is  $\leq 14$  days, suture removal is not necessary. The wound should be observed for swelling, heat, discharge, and opening of the incision (dehiscence) at least once daily until healed, and treated with an antimicrobial agent if an infection is noted. **NOTE:** The suture acquired from “MERCİ” may have expired and not be suitable for use for survival procedures.

### **Postoperative**

#### **Management**

1. Rodents should be kept warm with an external heat source both during surgery and afterwards until ambulatory. A heating pad underneath the drape or a heat lamp can be used. Be careful not to overheat the rodent when using a lamp. Monitoring rectal body temperature is the best method to ensure that the animal is neither hypo- nor hyper-thermic. Rodents under anesthesia become

essentially ectothermic and unable to control their body temperature. Hypothermia significantly prolongs anesthesia and impairs recovery.

2. An analgesic agent must be administered, preferably prior to recovery from anesthesia, unless exemption is granted by the ACUC in the protocol. Preemptive pain management is much more effective than giving medication after observing signs of pain. **Consult with a veterinarian when writing the protocol to devise the most appropriate pain management plan for your experiments.** In general, researchers have a choice of 3 drug classes: an opiate such as buprenorphine, butorphanol, or morphine; a nonsteroidal anti-inflammatory (NSAID) such as ketoprofen, meloxicam, ketorolac, or carprofen; and/or a longacting local anesthetic such as 0.25% bupivacaine. Buprenorphine works well for many surgical procedures because it provides analgesic blood levels for a period of 8 to 12 hours. Ketoprofen or meloxicam once every 24 hours is a good first choice NSAID. If systemic analgesia is not suitable for scientific reasons or if the surgical intervention is minimal and expected to produce only mild, short-lasting pain, local anesthesia such as bupivacaine (Marcaine) infiltration at the surgical incision may be an option. Oral dosing of any analgesic in the drinking water is generally not an effective method. Depending on the severity of trauma associated with the procedure, administration of an analgesic agent for up to 72 hours may be indicated.
3. Antibiotics are not necessary if sterile technique is used. However, some procedures warrant the use of perioperative antibiotics. This can be discussed with a veterinarian.
4. After surgery, once the animal can freely move around the cage, it may be returned to the vivarium. (Return to barrier facilities is not permitted. Check with the facility supervisor for appropriate locations.)
5. At least once daily, the animals should be observed for signs of pain, distress, and incision problems. The following list (from *Recognition and Alleviation of Pain and Distress in Laboratory Animals*, NRC 1992) provides various signs to be watched for that would indicate acute pain in rodents. For a more comprehensive treatise, refer to the Guidelines for Recognition of Pain and Distress on the ACUC Policy page at URL <http://www.virginia.edu/vpr/iacuc/policies.html>
  - a) Decreased appetite. May eat bedding or their offspring.
  - b) Decreased urine and fecal output.
  - c) Decreased activity.
  - d) Piloerection, ungroomed appearance.
  - e) Excessive licking and scratching that may progress to self-mutilation.
  - f) Abnormal stance or hunched posture.
  - g) Respiration can be rapid and shallow with grunting or chattering on expiration.
  - h) Pupils might be dilated.
  - i) Porphyrin secretion ("red tears") might be seen around the eyes and nose.
  - j) Vocalization.
  - k) Increased aggressiveness when handled.
6. Records must be kept in a laboratory notebook of all surgeries performed, anesthesia and analgesia administered, and any complications encountered. If any of the above signs of pain or distress are observed, a staff member in the Center for Comparative Medicine should be notified immediately so that veterinary care is provided. If the pain or distress is significant and intractable, the animal will be euthanized. Refer to the "Policy and Guidelines on Humane Endpoints" and "Policy on Recognition and Assessment of Pain and Distress". (ACUC Policy Page <http://www.virginia.edu/vpr/iacuc/policies.html>

## APPENDIX 5: POLICY ON TUMOR PRODUCTION & CANCER RESEARCH IN ANIMALS

Research animals are used to study tumor biology, develop and test new therapies, and evaluate potential carcinogens. Animals should be monitored frequently during tumor development to allow or intervention before significant deterioration of animal health or death occurs. Effective monitoring and endpoints should define limits on tumor burden and severity of tumor-associated symptoms. Alternatives to clinical signs are physiological, biochemical, and other biomarkers (such as anemia determined from hematocrits), in particular optical imaging of tumors labeled with reporter proteins or dyes. Growth of hybridomas in rodents is covered separately in the “POLICY ON ASCITES PRODUCTION IN MICE AND RATS”

### GENERAL GUIDELINES

1. For all cancer research animal models, **endpoints** must be established to minimize pain and/or distress, or scientific justification provided to do otherwise (Category E). The investigator should consult with a veterinarian and must include in the protocol plans for preemptive euthanasia based on defined endpoints (or address as Category E).
2. **Physical condition** of rats or mice inoculated with tumors must be assessed at least twice weekly and then daily near endpoints, including weekends and holidays. Records of observations must be kept and be available on request.
3. Animals with tumors that have ulcerated or that interfere with their ability to acquire food or water, or to maintain normal postural and ambulatory function, or animals that become emaciated or debilitated must be euthanized. If it is necessary to maintain an animal with an ulcerated tumor or some debility such as hind-end paresis, the status of the animal's overall condition must be assessed daily in consultation with a veterinarian, and appropriate steps taken to assess and minimize suffering.
4. Considering weight loss and gain (from growth), **tumor burden** should not exceed 10% of the animal's normal body weight (typically a subcutaneous tumor diameter of 16 mm in a 25 g mouse [or two 13 mm tumors] or 36 mm in a 250 g rat [or two 29 mm tumors]). *Tumors seldom grow as spheres*: a cylindrical tumor of 11.5 mm diameter will be 30 mm long when it reaches the 10% limit. Calibration curves should be established and posted for the guidance of staff, rather than relying on maximum size in one dimension. Volumetric calculations from 2 or 3 dimensions (width, length, and height) are better. See below for more information on tumor size calculation.

Rodent **weight loss** should not normally exceed 20% of body mass of equivalent tumor-free controls. For younger animals, failure to maintain weight gain to within 15% of untreated control animals may indicate drug toxicity or tumor effects. Baseline body weights must be recorded at the start of the project, and the weight of the animals must be recorded periodically to avoid exceeding the tumor burden and body weight guidelines above.

### INFORMATION FOR VIVARIUM STAFF AND ANIMAL HANDLERS

#### Recommended Endpoints:

- Tumor mass should not significantly interfere with normal bodily functions, or cause pain or distress due to its location (solid tumors)
- Weight loss, due to anorexia or cachexia, exceeding 20% of the body weight of a age and gender matched control (taking into account the tumor mass)
- Ulceration/infection of the tumor site
- Persistent self-induced trauma
- Progressive dehydration

- Hypothermia
- Lethargy
- Visible signs of anemia (e.g. pale mucous membranes)
- Piloerection (hair standing on end)
- Hunched posture or inability to move normally

Consult the IACUC Policy on Humane Endpoints for further details.

#### **Selected Clinical Observations Parameter What to look for:**

- General Appearance Dehydration, weight loss, abnormal posture, hypothermia, fractured limb, swelling, tissue masses
- Skin & Fur Discoloration, urine stain, pallor, redness, blueness, jaundice, wound, sore, abscess, ulcer, bald spot, ruffled fur
- Eyes Bug-eyed, microphthalmia, droopy lids, redevye, tears, discharge, opacity
- Nose, Mouth & Head tilted, nasal discharge, malocclusion, drooling
- Respiration Sneezing, rattle, abnormal breathing
- Urine Discoloration, blood in urine, excessive or no urination
- Feces Discoloration, blood in the feces, softness/diarrhea
- Genital area prolapse, paraphimosis
- Locomotion Hyperactivity, hypoactivity, impaired movement, coma, poor coordination, circling, tremors

Adapted from Montgomery, C.A. Jr. (1990), *Cancer Bulletin* 42:230237 and AWIC Newsletter, Spring 1995 6:4

**Appendix:** Subcutaneous tumor burden can be calculated from the formula for the volume of a spheroid:  $V = 4/3\pi(l/2)(w/2)(d/2)$ , where l is length, w is width; d is depth, measured with animal calipers. For a sphere, this is roughly  $1/2D^3$ , where D is diameter. It is not practical for vivarium staff to be doing mathematical calculations for tumor-bearing mice. **A sensible solution is for the investigator to provide a simple chart** with calculated maximum allowed dimensions for visible tumors, based on laboratory experience. The chart should be posted in the animal room to assist the staff with monitoring the well being of tumor-bearing animals. Since tumor mass is related to linear dimension to the 3<sup>rd</sup> power, the maximum diameter/width of two tumors is **80% each** relative to a single tumor.

## **INFORMATION FOR PRINCIPAL INVESTIGATORS AND PROTOCOL PREPARERS**

### **Biology of Tumors and Assignment to USDA Categories**

Cancer research in animals falls into two main classes:

1. Endogenous or spontaneous tumor formation, including carcinogen treatment. It is often unclear when tumor development initiates; animals with such tumors should be regarded in the same way as human patients and the animals classified in USDA Category E for pain and distress, unless the researcher will alleviate discomfort or has persuasive data on lack of discomfort due to the tumors.
2. Xenografts (usually of human cells). The animal may serve as a bioreactor for growth of the tumor. Such xenografts are often not innervated or known to cause pain. Subcutaneous tumor growth can be followed visually and may be classified as USDA Category C. Xenografts to internal sites and organs (e.g. orthotopic transplants) should be classified as USDA Category E, unless the investigator can justify otherwise. The Guidelines below list observable endpoints that correlate with responses to tumor. The limit for subcutaneous tumors of 10% of tumor-free body weight is based on appearance. Animals can be classified as USDA Category D, if they will be euthanized or treated with effective palliation when symptoms of distress appear.

***Intentional death is not an allowed end point, unless it is scientifically justified or a regulatory (e.g. FDA) requirement and approved by the ACUC. This applies both to toxicity studies and to therapeutic studies in animals bearing experimental tumors.***

In the case of tumors induced by carcinogens, viruses or genetic manipulation, factors such as method of induction may affect type and location of resulting tumors. Animals at risk of such tumors should be observed particularly frequently for signs of possible tumor development or associated disease. Biology of the tumor should be considered, include growth rate, invasion, distension, ulceration, metastases, site, and host cachexia for spontaneous and transplanted tumors. These features, which define the tumor profile, should be established in pilot experiments. Methods of tumor implantation or induction should be chosen so as to minimize trauma.

Contamination of tumor cell lines with human and/or rodent viruses and other microorganisms may compromise experiments or spread disease to other laboratory animals. Screening of cell lines for rodent viruses and mycoplasmas is recommended. For instance, tissue culture reagents are often contaminated with mycoplasma and LDEV, lactate dehydrogenase elevating virus (found in Matrigel), which can be transmitted from tumor to animal, although not to other animals, and can confound results of animal experiments. Human cell lines and tissue samples, including tumors, may carry and could shed unknown pathogens. Inoculation of human cells into animals must be approved by the **Institutional Biosafety Committee (IBC)** and declared in the animal protocol, including provisions for handling and waste disposal at **BSL2 safety level**, while carcinogens and cytotoxic chemotherapeutic agents are hazardous chemicals and should be handled accordingly.

### **Humane Considerations in Experimental Design**

**End points for tumor growth** need to be chosen carefully, based on experimental objectives and underlying biology, taking into account indicators of pain, distress, and deviation from normal behavior.

**Sites for tumor generation** should be avoided where tumor growth would cause pain or distress, or limit mobility. Subcutaneous or intradermal growth on the back or in the flank causes the least distress, while implantation of tumors in the footpad, tail, brain and eye requires special protocol justification. Distension of musculature is generally painful; this should be considered with intramuscular implants. Extra attention must be paid if multiple sites are used. One or two larger tumors can be more accurately measured than many small ones and yield data with greater statistical significance.

- Tissue **necrosis or ulceration** of the skin overlying the developing tumor may occur.
- Ulceration can occur when tumors develop subcutaneously or are inoculated into the derma. Ulceration or necrosis may result in loss of body fluids and/or infection, requiring euthanasia.
- In the case of **leukemias**, internal, disseminated, and metastatic or other occult tumors, determination of the tumor burden may be difficult. Biochemical serum markers or pathology data from a pilot experiment should be used. Development of **spontaneous tumors** in all transgenic animals and in those strains known to be cancer-prone should be monitored, with examination of both predicted and unexpected sites for tumor development, including weight changes, palpation, and monitoring for deterioration in clinical condition. Experience suggests that animals should be examined at least twice weekly throughout their lifespan.
- Appropriate assessment includes: evaluation of overall clinical condition (including appearance, posture, body temperature, behavior and physiological responses), assessment of food and water intake; Weighing (both positive and negative changes compared to controls can be associated with increasing tumor burden); Caliper measurements to determine tumor volume; and inspection and palpation to locate the sites of tumor growth, as well as to assess distension, ulceration and compromised mobility. Special techniques may be valuable for

specific sites, e.g. respiratory rate for lung involvement, neurological disturbance or irreversible weight loss for brain neoplasms, and blood cell counts for

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## APPENDIX 6: POLICY ON GENOTYPING OF MICE BY TISSUE BIOPSY

### Introduction

To determine the genotype of genetically engineered mice (GEM), it is necessary to obtain a tissue biopsy, isolate DNA from the biopsy, and subject the DNA to molecular biological testing such as polymerase chain reaction (PCR), Southern analysis, or dot/slot blot hybridization. This is commonly performed at the time of weaning by obtaining a small piece of the most distal portion of the tail and at the same time giving the mouse unique identification. The purpose of this policy is to establish standards for obtaining tail biopsy material while minimizing pain and distress to GEMs.

A recent publication compared five strains of inbred mice to determine the age of coccygeal vertebrae ossification, the order of coccygeal vertebrae ossification, and the behavioral response to tail biopsy.<sup>5</sup> This publication demonstrated that: mature tail vertebrae (i.e., ossified vertebral body and growth plates) in the distal 5 mm of tail were apparent in most strains using micro-computed tomography by 17 – 21 days of age (34-38 days when evaluated by micro-radiography); that all strains showed an acute behavioral response during the initial 10 minutes after biopsy that diminished by 60 minutes post-biopsy ( $\leq 10$  percent in mice 17-28 days of age compared with  $\leq 40$  percent in mice 31-42 days of age 60 minutes post-biopsy); and that the distal 5 mm tail vertebrae ossified several days later than those more proximal to the body. This article concluded that a 5 mm tail biopsy in mice no older than 17 days of age could be performed without anesthesia whereas older mice required anesthesia. Similar studies in mice using telemetry of heart rate concluded that tail biopsy was no more stressful than manually restraining mice.<sup>6</sup>

### Regulations and Guidelines

While the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (1996, National Academy Press, Washington, DC) does not address this issue, the intramural NIH program has adopted guidelines pertaining to this topic. These guidelines, however, do not bind awardee (extramural NIH funded) institutions.

### Alternatives to Tail Biopsy

There are several sources of tissue that can be used to obtain DNA for the purpose of genotyping mice. Such sources include buccal cells obtained by oral swab or fecal material contain sloughed gastrointestinal cells; however, in studies published using this method the low DNA yield requires a nested PCR, which may lead to false positives due to contamination of the laboratory with amplified DNA.<sup>1,3</sup> Another source of DNA is an ear punch biopsy. When this method of identification is used, care must be taken to adequately remove residual ear tissue from the ear punch as this can confound results of subsequent biopsies.<sup>2</sup>

### Biopsy Policy

The following UVa ACUC policy is based upon: post-biopsy behavioral and telemetry data in the literature, data on age of ossification of distal tail vertebrae, routine GEM colony management practices, and years of GEM tail biopsy observation:

- The biopsy procedure must be included in an approved protocol.
- If one of the alternatives to tail biopsy above is suitable for the laboratory, it can be used without anesthesia (local or general) in mice of any age.
- If a tail biopsy is required, mice should be no older than 28 days. A piece of tail 5 mm from the distal end should yield a sufficient amount of DNA for PCR, dot blot, or slot blot analysis. If mice older than 28 days are biopsied, they must receive analgesia either topically or systemically. Recommended analgesics are listed in this policy.
- If more than 5 mm of tail is to be biopsied, or a single mouse needs to be biopsied more than one time, then the use of a local or general anesthetic is required.

- Options for local anesthesia include: topical ethyl chloride spray, topical 1% lidocaine in 50% dimethylsulfoxide, or submerging the tail in ice-cold ethanol for 10 seconds.
- In all cases, tails should be disinfected with 70% ethanol prior to the biopsy and monitored for hemostasis after the biopsy. In some cases, bleeding may need to be controlled; provide hemostasis by digital pressure and/or application of a styptic agent (e.g., Clotisol® or silver nitrate).

### **Instructions for the Use of Ethyl Chloride**

Ethyl chloride spray is available from the Center for Comparative Medicine. It comes in a glass bottle that would represent a hazard to the user if accidentally broken in one of the small vivarium rooms since it rapidly evaporates when spilled. It is highly explosive and should not be used near an open flame or devices that spark (e.g. an electric motor). **There are no serious health hazards in connection with its occasional clinical use as vapo-coolant, provided it is used in a well-ventilated area.**

A small aliquot of ethyl chloride is transferred into a microcentrifuge tube for use in the mouse room. Use the microcentrifuge tube of ethyl chloride in the following manner:

1. In a chemical fume hood, invert the ethyl chloride bottle and spray 1 ml into a 1.5 ml polypropylene microcentrifuge tube (either snap or screw top) for use in the mouse room.
2. In the mouse room, place the 1.5 ml tube with ethyl chloride into a stable stand.
3. Put unique identifier on mouse (ear punch or ear tag).
4. Submerge the tip of the mouse's tail to be amputated into the ethyl chloride for 1-2 seconds.
5. Remove the tail from the liquid.
6. Amputate 5 mm or less of the distal tail tip into a tube labeled with the mouse identification.
7. Control bleeding if necessary by digital pressure or styptic (Clotisol) application.
8. Return mouse back into cage.
9. If you are going to pause for a period of time, cap the ethyl chloride tube to prevent evaporation.

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**APPENDIX 7: ANIMAL TRANSFER BETWEEN PROTOCOLS AND/OR DELIVERY FORM**

**BASIC INFORMATION SECTION**

Date Form Completed:  Principal Investigator:

Principal Investigator's e-mail:  Phone number:

Species:  Strain:

IDs: (Sex, pen #, tag # etc.)  Biohazardous (ABSL-2):  Yes  No

No. of animals to be moved or transferred:  No. of cages to be moved or transferred:

**Building animals sent from:**  **Room Number:**

**Protocol Animal is currently under:**

Per Diem Fund Code:

\_\_\_\_\_  
Principal Investigator's Signature Date

Section One: PROTOCOL TRANSFER REQUEST

**Building Animals to be housed in:**

Building:  Room Number:

**Protocol Animal is being transferred TO:**

Principal Investigator:

Per Diem Fund Code:

**Section Two: DELIVERY REQUEST**

Date Delivery Needed:  Time Delivery Needed:   AM  PM

Deliver to: Building:  Room Number:

*\*Animals must be fully alert in order to be returned to animal housing*

Return transport to originating facility and room?  Yes  No. If Yes, Pick-up time:

How will cages be marked for transport?

## INSTRUCTIONS FOR COMPLETING FORM

- Everyone should complete the BASIC INFORMATION SECTION at the top of the form.
- If you are only **transferring** an animal(s) from one protocol to another, complete **Section One**.
  - **Animals that have had any experimental manipulation may not be transferred between animal protocols.**
  - **Only the signature of the principal investigator will be accepted for transfers between animal protocols.**
  - **ABSL2 cages must be enclosed in a paper or plastic bag and labeled with the biohazard symbol.**
- If you are requesting **delivery only** of an animal(s) from one place to another complete **Section Two**. 24 hours notice and veterinary approval from the Director of Comparative Medicine is required for delivery of an animal(s) from one vivarium to another, e-mail shf2b@virginia.edu and state where they are coming from and going to.
- If you are requesting **transfer** of an animal(s) from one protocol to another **and** also requesting **delivery** to another location **complete both sections one and two**.
- Fax to the completed form to 4-0354.

# Appendix 8:UVA Center for Comparative Medicine Drug Request Form

Fax #: 924-0354

Comparative Medicine Staff Only Filled Date: _____ Initials: _____
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Orders must be submitted by fax or in person to the Comparative Medicine office in MR5 at least 3 business days in advance. (revised 02/06/09)

Name: \_\_\_\_\_  
 Phone: \_\_\_\_\_  
 \*Email: \_\_\_\_\_  
 PI: \_\_\_\_\_

Date: \_\_\_\_\_  
 Vivarium Animals are Housed \_\_\_\_\_  
 Protocol: \_\_\_\_\_

**Controlled Drugs** Quantity Btl MI

Ketamine 100mg/ml				
Pentothal 1gram				
Nembutal 50mg/ml				
Euthanasia				
Mouse Anesthetic Mix				
Rat Anesthetic Mix				

**Non-Controlled Drugs** Quantity Btl MI

Xylazine 20mg/ml				
Xylazine 100mg/ml				
Mouse Ketoprofen Mix				
Rat Ketoprofen Mix				
Ketoprofen 100mg/ml				
Atropine 0.4mg/ml				
Bupivacaine 0.25%				

**Controlled Drugs** # amps

Buprenex 0.3mg/ml	

**Non-Controlled Drugs** # Btl.

Isoflurane	
Domitor	
Antisedan	
Heparin 1000 units/ml	
Nexaband (Surgical Glue)	
10ml Saline Bottle	
Ethyl Chloride	
Clotisol	
Neo-Predef Powder	
Puralube (Eye Ointment)	
Baytril 22.7mg/ml	

**Controlled Drugs** # patches

Duragesic 25µg	
Duragesic 75µg	

**Special Requests and/or Comments:** \_\_\_\_\_

If you have an emergency, page Gina Wimer 1845, Jeremy Gatesman 4145 or Linda McVay 3680.  
 You may be charged a \$10.00 I.D.P.A (I Didn't Plan Ahead) Fee.

Forms are available online at [http://www.healthsystem.virginia.edu/internet/ccm/forms\\_and\\_documents.cfm](http://www.healthsystem.virginia.edu/internet/ccm/forms_and_documents.cfm) or in the Center for Comparative Medicine office.

Appendix 9:

**UNIVERSITY OF VIRGINIA  
CENTER FOR COMPARATIVE MEDICINE**

SOP#: 211 APPROVED \_\_\_\_\_ DATE \_\_\_\_\_  
Attending Veterinarian

Date Issued: Original

Date Revised: 11/21/03

Page 1 of 1

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**TITLE:** Proper Use of Carbon Dioxide Chamber for Euthanasia

**SCOPE:** Veterinarians, Veterinary Technicians, Animal Care Staff, Researchers

**RESPONSIBILITY:** Veterinary Staff

**PURPOSE:** To outline procedure for euthanizing rodents using carbon dioxide

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**Background information on the use of CO<sub>2</sub>:** Exposure to CO<sub>2</sub> at high concentrations causes rapid unconsciousness and anesthesia. It is a well accepted and commonly used method of euthanasia for small mammals and birds. It is cost-effective, nonflammable, and nonexplosive. It presents minimal hazard to personnel when used with properly designed equipment. It causes no accumulation of chemical residues in tissues. It does not distort cellular architecture except rat lungs. One disadvantage of CO<sub>2</sub> is the increased time needed to euthanize neonates due to increased resistance to hypoxia in these animals (use an alternative method).

**PROCEDURE:**

1. Check chamber for proper connections of hose from CO<sub>2</sub> tank to chamber and for open outlet at the top of chamber for release of displaced air. Check regulator gauge on CO<sub>2</sub> tank to make sure you have adequate amounts of CO<sub>2</sub> left in the tank prior to beginning (full tank = 750 psi).
2. Prefill chamber with CO<sub>2</sub> by opening the valve that directs CO<sub>2</sub> into the chamber. Let fill for 1 minute at a rate where filling is audible as a low volume hiss (5-8 psi, 4-6 L/min)..
3. Open lid and place animal/animals in chamber. They can be left in a mouse box and place the whole box in the chamber or placed directly in the chamber.
4. Continue filling the chamber for 3-5 more minutes at a slow rate (an optimal flow rate should displace approx. 20% of the chamber volume per minute).
5. Turn off the CO<sub>2</sub> once the animals appear unconscious. Clamp outflow tubing or plug opening to retain CO<sub>2</sub> in chamber. Keep the chamber closed until all respirations have ceased. In some animals, this may take as long as 5 minutes. Neonates (pups up to 7 days old) should be left in the chamber for at least 10 minutes. (Euthanasia of neonates with an overdose of halothane is preferred to using CO<sub>2</sub>).
6. **Verify death of the animals after removal from the chamber by palpating the chest and confirming absence of a heartbeat. If a heartbeat is still detectable, the animals should either be reintroduced into the chamber or euthanized by an alternative method. Cervical dislocation under anesthesia or decapitation of neonates is an acceptable method of euthanasia.**

# University of Virginia Center for Comparative Medicine

## Barrier Techniques Training

Name (printed): \_\_\_\_\_ Department: \_\_\_\_\_

UVA 9-Digit Assigned #: \_\_\_\_\_

IACUC Protocol Number(s): \_\_\_\_\_

Office or Lab Location: \_\_\_\_\_

Office and / or Lab Telephone #: \_\_\_\_\_

Alternate Telephone # (pager or cell): \_\_\_\_\_

E-mail address: \_\_\_\_\_

DOCUMENTATION OF BARRIER TRAINING

**Vivarium Access Control**

- Vivarium practices
- Animal Orders from Commercial Vendors
- Aseptic Techniques for Rodent Cage Changing and Other Barrier Room Practices
- Rodent Diets, Drinking water, Bedding, and Specialized Caging
- Movement of mice out of barrier rooms and the vivarium
- Euthanasia and carcass disposal
- Technical Support-Time and Vet-tech Time
- Special cage card designation - Animal surgery cards (Post Operative), Animal treatment (Sick) cards
- Vivarium specific information
- Experiments involving biosafety level 2 organisms

_____	_____
Printed Name of Trainer	Initial
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
Printed Names of Personnel Trained	UVa e-mail ID