Basic MOUSE Handling and Technique Guide
UNC-IACUC

“First do no harm” – Greek Hippocratic Oath, Great Watch Words of Medicine

Basic Information about working with Mice

Proper Personal Protection Equipment (PPE) is a requirement for working with animals. In DLAM facilities, minimum requirements include disposable coveralls, shoe covers, head bonnet, mask, and gloves. Please review requirements before entering any animal area! Requirements may change from room to room so each door is posted with instructions. The use of a face mask reduces your risk of allergy to animals. We strongly recommend that you wear masks whenever you work with animals.

If Bitten: *DO NOT PUNISH THE MOUSE FOR ITS NATURAL RESPONSE*

1. Calmly return the animal to its cage
2. Wash the wound with antibacterial soap and water
3. Bandage the wound
4. Notify your supervisor and contact the University Employee Occupational Health Clinic (UEOHC) 919-966-9119

Training Information

*Training and certification may be obtained in two ways:*

1. Each laboratory must designate a Laboratory Animal Coordinator (LAC) who may train research personnel in their laboratory in various animal-handling techniques. The LAC must be certified by the Office of Animal Care and Use (OACU) or the Division of Laboratory Animal Medicine (DLAM) and demonstrate proficiency before training others within their lab. Please note, there are certain techniques that LAC’s are not allowed to train in.

2. For additional training please contact the Training and Compliance Coordinators for the Institutional Animal Care and Use Committee (IACUC) at 966-5569. We offer training in both one on one and classroom settings. For a look at IACUC Guidelines and dates of future classes, visit our website at: http://research.unc.edu/offices/iacuc/training/

Network of Laboratory Animal Coordinators (NLAC)

Also, consider joining the Network of Laboratory Animal Coordinators (NLAC) listserv. This listserv is an internal avenue for laboratory animal coordinators at UNC to make contacts, exchange ideas, ask questions, and make suggestions. The goal is to enhance communication between animal research laboratory personnel, the Institutional Animal Care and Use Committee, and the Division of Laboratory Animal Medicine. See the following link for more information and to subscribe: http://research.unc.edu/offices/nlac/
Mouse Behavior and Health

1. Mice are usually mild in temperament and easy to handle. They are not usually aggressive, but can bite if frightened. There are some strains that are aggressive and can inflict painful bites.

2. Mice are nocturnal animals. Activities such as eating, drinking or mating are typically done at night.

3. Mice groom themselves almost constantly to maintain a smooth, glossy haircoat (Figure 1). A ruffled or dull haircoat is generally a sign of illness or distress (Figure 2).

4. Dominant mice exhibit a behavior called barbering. Barbering is the dominant mice biting or chewing on the fur of a more subordinate mouse. Barbering should not be confused with fur loss due to illness. Typically, barbering occurs around the face or back.

5. Male mice can be more aggressive and fight more often than females. Aggressive mice should be housed individually to avoid severe injury to cage mates. Generally male littermates may be housed together, but once separated, it is advisable to only house males with females.

6. Mice are creatures of habit. Everyday events do not tend to stress or excite the mice. However, handling and restraint can be stressful and result in the mouse being difficult to work with. Conditioning the mice to such handling (so they do not associate handling and restraint aversively) can make the animals much easier to work with.

7. Check rodents’ teeth frequently. This will insure early detection of malocclusion. If maloccluded, teeth may become overgrown and interfere with eating (Figure 3). Scissors can be used to trim maloccluded teeth (Figure 4).

**Figure 1: Healthy Mouse**

**Figure 2: Unhealthy Mouse**

**Figure 3: Malocclusion**

**Figure 4: Malocclusion Scissors**
Sex Determination
Gender in mice is determined by comparing anogenital distance, or the distance between the urogenital opening and the anus. Male mice typically have a larger anogenital distance when compared with the females. Be aware there are variances in anogenital distance among strains. See Figures 5 and 6 below.

Figure 5: Sex Determination in Mice

![Adult female mouse. Note shorter anogenital distance and vulva marked by black line](image1)

![Adult male mouse. Note greater anogenital distance marked by black line](image2)

Figure 6: Sex Determination in young mice

![Buck (9 days)](image3)

![Doe](image4)

*Note visible teats on the young female mouse (R) and lack of teats on male mouse (L).
Handling and Restraint

When picking up adult mice, grasp them gently but firmly at the base or center of their tail. Do not pick them up by the tip of the tail. Place the animal on a surface such as the wire cage top or lid (Figure 7a). It is best that the surface not be slick or smooth as mice will behave much more calmly if they have firm footing. While still holding the tail near the base, with your other hand firmly grasp the loose skin on the back starting near the ears using your thumb and first two fingers (Figure 7b). The tail can then be held by the last two fingers as shown (Figure 7c). Your grip should be firm enough to keep the mouse from struggling, but gentle enough for it to breathe comfortably.

For quick handling, such as cage to cage transfers, it is acceptable to use forceps (Figure 8). Gently grasp the loose skin on the back and quickly transfer them to the new cage. This technique is useful for fractious or aggressive animals. Be sure to clean gloves or forceps with a disinfectant such as Virkon between cages.

Another method of transferring mice is referred to as the “tube” or tunnel method (Figures 9a & 9b). For this method, gently guide the mouse into the tunnel with one hand while holding the tunnel with the other. Once the mouse enters the tunnel, gently tilt it and close your hands over the tunnel. Transport the mouse to the destination cage and release. This method is recommended for more anxious strains of mice.

Gloves, either light leather, cloth or mesh greatly diminish sensitivity, increasing chance of injury to mice. They also make it more difficult to perform delicate procedures, and mice can often bite through them.
Examples of Commercial Restrainers:

Various styles and sizes available: See Page 24 for vendors

Snuggle
Lomir.com

Tail vein restrainer
braintreesci.com

Flat bottom restrainer
braintreesci.com

Mouse Injections

Basics and a few tips

- Use a fresh, sterile needle for each injection; do not reuse needles between animals
- To avoid excessive leaking, keep the needle in the needle tract for a few seconds following injection
- Always inject with the bevel of the needle facing up
- When giving substances intravenously, inject slowly to avoid shock.
- Injecting slowly when giving substances intramuscularly will cause the least amount of pain.

Figure 10: Parts of the Syringe and Needle

Table 1: Recommended Volume and Needle Gauge by Injection Type

<table>
<thead>
<tr>
<th>Injection Type</th>
<th>Ideal Needle Size (Gauge)</th>
<th>Recommended Volume</th>
<th>Maximum Volume (in ~ 25 g mouse)</th>
<th>Aspiration Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraperitoneal (IP)</td>
<td>25-27G</td>
<td>20 ml/kg</td>
<td>60 ml/kg (1.5 ml)</td>
<td>No</td>
</tr>
<tr>
<td>Subcutaneous (SQ, SC)</td>
<td>25G</td>
<td>10 ml/kg</td>
<td>40 ml/kg (1 ml)</td>
<td>Yes; inject if no blood in the needle hub</td>
</tr>
<tr>
<td>Intramuscular (IM)</td>
<td>27G</td>
<td>25 μL per site</td>
<td>50 μL per site</td>
<td>Yes; inject slowly if no blood in the needle hub</td>
</tr>
<tr>
<td>Intravenous (IV)</td>
<td>26-28G</td>
<td>2.0-4.0 ml/kg/hr</td>
<td>5.0 ml/kg (100 μl)</td>
<td>No; inject slowly</td>
</tr>
</tbody>
</table>

Adapted from “Formulary for Laboratory Animals, 3rd, Ed. Hawk, Leary and Morris, 2005.”
1. **Intraperitoneal Injections**

This injection is made into the abdominal cavity, penetrating the peritoneum of the animal. It is important to avoid the midline to prevent penetrating the bladder.

- Scruff the mouse firmly and hold in dorsal recumbency (*Figure 11*).
- Insert the needle bevel up in a position below the bend of the knees; to the left or right of the midline (*Figure 12*).
- Angle the needle approximately 30°-45° to the body and inject (*Figure 11*).

*Figure 11: IP Injection Restraint*  
*Figure 12: Target IP Injection Site*

2. **Subcutaneous injections**

This injection is administered beneath the skin and above the underlying muscle (*Figure 14*). It may be performed in any area of loose skin along the back or flank.

- Firmly scruff the mouse and create a skin tent between the shoulder blades or over the flank (*Figure 13*).
- Insert the needle into the pocket and aspirate prior to injection to ensure the needle is properly positioned. If no blood is seen in the syringe, proceed with the injection.
- The outline of the needle should be clearly visible under the skin when correctly situated.

*Figure 13: Subcutaneous Injection*  
*Figure 14: Subcutaneous Injection Site*
3. **Intramuscular injections**

This injection is made into the posterior thigh of the mouse. It is only used in instances where the other injections are not appropriate, since it is potentially more painful than other injection routes.

- The muscle mass running along the back of the leg is used (*Figure 15*). Two people or a restrainer are usually required for the technique.
- If using the two person method, one person should firmly restrain the mouse and the second person extends the rear leg and performs the injection (*Figure 16*).
- If using a restrainer, gently pull the mouse into the restrainer as normal, but pull the leg through the top. Be careful to not dislocate or twist the leg (*Figures 17a & 17b*).
- Insert the needle angled parallel to the femur and pull back slightly on the plunger to ensure the needle is not in a blood vessel (*Figures 16 and 17b*). Proceed with the injection if no blood is aspirated. Do not inject if blood is aspirated.
- Care must be taken to avoid the sciatic nerve and the femoral vein, artery and nerve.

*Figure 15: Intramuscular Injection Site*  
*Figure 16: Intramuscular Injection - 2 people*  
*Figure 17a: Intramuscular injection with restrainer*  
*Figure 17b: Intramuscular injection with restrainer*
4. **Intravenous injections**

This injection is done into the lateral tail veins of the mouse. It is one of the most difficult injection techniques to master.

- Place the mouse into a restrainer and dilate the blood vessels by warming them. Warm water, a heat lamp, or hand warmers may be used for this purpose.
- Locate the dorsal vein. When the mouse is in an upright position it will be near the 12’ o clock position. Mark the dorsal vein with a marker, and then locate the left and right lateral tail veins, in the 10 o’ clock and 2 o’ clock positions (*Figure 20*). Injections can be made into either of these veins.
- Insert the needle bevel up as low as possible towards the tip of the tail and with the needle positioned horizontally to the vein. The vein is very superficial and requires the needle to be inserted with very little angle to penetrate the vein (see *Figures 18 & 19 below*).
- The vein will clear from the injection site to the base of the tail if properly situated, whereas ballooning around the injection site will occur if the needle is inserted subcutaneously.
- If you suspect the injection was subcutaneous, withdraw the needle and try again moving up the tail towards the base or using the other lateral vein. Note that as you move up toward the base of the tail the vein is located more deeply.

*Figure 18: Cross Section of Mouse Tail*

*Figure 19: Intravenous Injection*

*Figure 20: Lateral Tail Veins*

10 o’clock & 2 o’ clock position
5. Oral Gavage

Oral gavage allows for gastric delivery of substances. A stainless steel, ball tipped gavage needle is recommended. It is very important the needle be the appropriate size and that you measure prior to administration to ensure that you insert the needle to the proper depth. If the tube is too short, the injected fluid may be aspirated by the mouse causing possible pneumonia and death. If the needle is too long, it may perforate the stomach. Improper gavage technique can cause tearing of the esophagus or asphyxiation. Oral dosing should not exceed more than 10ml/kg.

- Firmly restrain the mouse and measure the needle before gavaging. The tube should measure the distance from the tip of the nose to the last rib, so that the needle will pass down the esophagus into the stomach (Figure 22).
- Insert the gavage needle into the mouth at one side at about a 45° angle. Slide the needle down the back of the throat while tilting the mouse’s head back, so that the neck is in a straight line (Figure 21).
- The needle should pass easily down the esophagus; with little to no resistance. If the mouse struggles or resistance is met, stop, withdraw the needle and start over.
- Observe the mouse carefully after the gavage is completed. No fluid should be coming from the mouth or nose and the mouse should not show signs of distress.

Table 2: Gavage Needles - Appropriate Size and Volume by Mouse Weight

<table>
<thead>
<tr>
<th>Mouse Wt (g)</th>
<th>Gauge</th>
<th>Length (inches)</th>
<th>Ball Diameter (mm)</th>
<th>Recommended Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 14g</td>
<td>24</td>
<td>1</td>
<td>1.25</td>
<td>~ 1 % of BW</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.14 ml for 14 g</td>
</tr>
<tr>
<td>15-20g</td>
<td>22</td>
<td>1 or 1.5</td>
<td>1.25</td>
<td>0.15-.20 ml</td>
</tr>
<tr>
<td>20-25g</td>
<td>20</td>
<td>1, 1.5 or 3</td>
<td>2.25</td>
<td>0.20-0.25 ml</td>
</tr>
<tr>
<td>25-30g</td>
<td>18</td>
<td>1, 1.5 or 2</td>
<td>2.25</td>
<td>0.25-0.30 ml</td>
</tr>
<tr>
<td>30-35g</td>
<td>18</td>
<td>2 or 3</td>
<td>2.25</td>
<td>0.30-0.35 ml</td>
</tr>
</tbody>
</table>

*Source for recommended sizing: Braintree Scientific

Figure 21: Gavage

Figure 22: Measurement for Gavage
Anesthesia Injectables
Anesthesia injectables may be utilized for survival surgeries. Please see the IACUC Anesthetized Procedure Guidelines for Rodents for more details about IACUC expectations for surgical procedures: https://research.unc.edu/files/2017/02/IACUC-Anesthetized-Procedure-SOP-for-Rats-and-Mice.pdf

- Weigh animal prior to anesthesia administration to ensure appropriate dosage.
- Inject animal with approved anesthesia dosage via the approved route. Note, anesthesia must be pharmaceutical or veterinary grade and not expired.
- Animal should lose consciousness slowly. Wait until the animal appears to be fully unconscious and is breathing slowly and steadily before removing from cage.
- Pick up animal and check for blink reflex. If no blink reflex is present, ophthalmic ointment must be applied. Failure to apply the ointment may result in irritation, infection, and corneal ulcers.
- Check for toe pinch reflex. In order to do this, take the animals paw between your thumb and index finger and pinch firmly, but not so hard as to hurt the animal. If the animal withdraws the foot, it is still able to feel pain and you should either wait or re-dose with anesthesia according to the approved protocol. If there is no response, you may begin surgery.
- Set animal on an appropriate heat source, including the tail, with care taken to avoid overheating and burns from heat sources. This is important because animals under anesthesia lose the ability to thermoregulate.
- Anesthetized animals must be observed at all times during anesthesia (including induction and recovery) and not left unattended until ambulatory.
- Return animal to animal room only when fully awake and ambulatory.
Documentation of Anesthesia

Dates and times of all observations, treatments, and procedures must be recorded (including anesthetic and confirmation of appropriate anesthetic depth). Date of procedure, procedure name, and date/time of postoperative analgesics/observations must be recorded at the cage level. Any deviations from the procedure as approved in the protocol must be reviewed by a DLAM veterinarian prior to implementation and then amended to the appropriate protocol and approved by the IACUC. All records must be available for review at any time by IACUC representatives and external regulatory officials. If the cage card will supplement your primary documentation record, than this card must be kept for the life of the IACUC protocol. Please see the example template for documenting drug use, available on the IACUC website: https://research.unc.edu/files/2012/11/Drug-Log-Template.pdf

*Note - If controlled substances are used they must also be recorded in the controlled drug log per DEA requirements.

Figure 25: Example Drug Log Template

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Controlled substance: Yes / No</th>
<th>Concentration, dose &amp; route</th>
</tr>
</thead>
<tbody>
<tr>
<td>(e.g. 100mg/kg)</td>
<td></td>
<td>(e.g. 10% solution)</td>
</tr>
</tbody>
</table>

Expiration date

<table>
<thead>
<tr>
<th>Date</th>
<th>Protocol #: Species</th>
<th>Animal ID / Group ID</th>
<th># in group</th>
<th>Total Volume Injected (ml)</th>
<th>Amount Waste (ml)</th>
<th>Amount Left in Bottle</th>
<th>Initials</th>
<th>Toe Pinch</th>
<th>Procedure &amp; Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>
**Rodent Identification**

There are several IACUC approved methods used to identify rodents. Please see the following document on the UNC IACUC website for more detail on a variety of identification methods, as well as the advantages and disadvantages to each method:

**Identification Methods for Rodents**: [https://research.unc.edu/files/2015/05/Rodent-Identification.pdf](https://research.unc.edu/files/2015/05/Rodent-Identification.pdf)

1. **Ear Notch**

   This method is frequently used in both mice and rats. There are several tools that may be purchased to achieve this (*Figure 26*). Most resemble a hole puncher and are very cheap. There are previously created ear punch maps that serve as a numbering system, or the researcher may create a map.

   - Firmly scruff the mouse and visualize the ear.
   - Place the flat side of the ear notch tool on the outside of the ear so that you can see the area you will be punching. Aim for the outer part of the ear to avoid the vasculature and excessive bleeding (*Figures 27a & 27b*).
   - Firmly and quickly press down and punch the ear, maintaining a firm restraint on the animal to avoid being bitten.

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*Figure 26: Ear Notch Tools*

*Figure 27a: Ear Notch – Short Tool*  
*Figure 27b: Ear Notch – Long Tool*
2. Ear Tag

Ear tags can be purchased with numbers and/or letters. Correct placement of the tag makes them easy to read. Note, proper placement of the tag is important. If the tag is placed within the vasculature of the ear (too far inward) the mouse may experience itching and irritation, which can lead to infection. If the tag is not fully closed, the mouse may also get its front foot caught in the tag. To avoid these issues, be sure to fully close the tag and place it in the outer part of the ear. See Figures 28-30 below demonstrating an open and closed tag.

- Place the ear tag flat on the back of your hand, and gently collect the tag with the ear tag tool (Figure 28).
- Firmly scruff and restrain the mouse in your non-dominant hand.
- Position the ear tag into the desired position. Aim for the outer ear avoiding the vasculature and towards the lower half of the ear so that the tag hangs appropriately (Figure 29).
- Firmly close the ear tagging tool until the tag is closed (Figure 30).

*Figure 28: Correct Ear Tag Placement in Ear Tagging Tool*

*Figure 29: Correct Ear Tag Placement in Ear*

*Figure 30: Fully Closed and Correctly Placed Ear Tag*
Please note other forms of identification not included here:

Long-term
- Microchip
- Tattoo
- Toe clips (see IACUC policy: http://research.unc.edu/files/2013/10/Toe-Clip-Policy-rodents.pdf)

Short-term
- Hair clip
- Permanent marker and fur dye

If you would like to inquire about equipment used in methods discussed above, please email the OACU Training/Compliance Team. You may send questions to the general IACUC email account iacuc@med.unc.edu
Blood Withdrawal Techniques

A variety of blood withdrawal techniques may be used in laboratory mice. The chosen route of blood collection should minimize pain and distress to the animal as much as possible while still achieving study objectives.


- **Chronic Blood Withdrawal**: For sequential blood sampling (over a period of time), the maximum survival blood withdrawal for most mammals is 1.5% of lean body weight every 14 days. If blood is needed weekly, 0.5% body weight is a safe amount.

- **Acute or Single Blood Withdrawal**: The maximum survival amount of an acute blood withdrawal is 1% of the lean body weight. [e.g.; For a 20 gram adult mouse, no more than 4 x 50 ul micro capillary tubes (200 ul), may be withdrawn]. The volume of blood in an adult mouse is about 78-80 ml/kg. This is approximately 10% of its body weight. Only about half of this can be recovered in a terminal blood withdrawal procedure.

1. **Submandibular bleed**

   A relatively simple way to obtain blood from a mouse is to puncture the area behind the hinges of the jawbones. A mouse bleeding lancet is strongly recommended for this technique. However, an 18 gauge needle may also be used. More information on lancets and a video of this procedure may be found at the following website: [http://www.medipoint.com/html/animal_lancets.html](http://www.medipoint.com/html/animal_lancets.html)

   - Scruff the mouse firmly and be sure that the head is restrained.
   - Align the lancet caudally to the mandible and locate the hairless area on the mouse (more easily seen in white mice). The puncture site is slightly behind this area (Figure 33).
   - Firmly insert the lancet perpendicular to the face and collect the blood with a tube (Figure 34).
   - Be careful not to cut too close to the ear, it is possible to perforate the ear drum. This is evident when the mouse begins to bleed from the ear. If this happens, it must be humanely euthanized.

![Figure 33: Submandibular Vein](image)

![Figure 34: Submandibular bleed](image)
2. **Saphenous Vein**

This method of obtaining blood is often used when a series of small samples is required. This method of blood withdrawal does not require anesthesia; however, the method of restraint is cumbersome.

- Place the mouse in a conical tube and shave the caudal surface of the thigh. The saphenous vein can be seen in this area.
- Apply a lubricant or shave the leg to prevent wicking. Place a tourniquet above the knee and enter the vein with a 25 gauge needle.
- Micro-hematocrit and microvette tubes may be used to collect the blood.

![Figure 35: Saphenous Vein](image)

![Figure 36: Saphenous Blood Collection](image)

3. **Tail Artery / Vein (NICK)**

Tail veins and artery can be used for serial bleedings. Use the central tail artery or lateral tail veins. Anesthesia is not required for tail nick.

- Place the mouse in a restrainer or have another person firmly restrain the animal (Figure 37a).
- Start midway up the tail and nick the artery or vein with a needle or lancet (Figure 37b).
- You may collect blood with micro capillary tubes, a micropipette or various microtainer collection tubes. Move cranially 0.5 cm at a time applying pressure after the bleed.

![Figure 37a: Tail Nick](image)

![Figure 37b: Tail nick](image)
4. **Tail Clip Bleed and/or Tail Biopsy for Genotyping**

The IACUC has approved the tail cut method for both rats and mice to obtain blood and/or tissue. This method must be described in the animal use application and approved by the IACUC prior to use. It may be performed on un-anesthetized or anesthetized animals depending on amount of tissue needed. Please see the following document for IACUC guidelines on tail clips: [http://research.unc.edu/files/2012/11/Guidelines-for-Rodent-Blood-Withdrawal-and-Tail-Biopsy.pdf](http://research.unc.edu/files/2012/11/Guidelines-for-Rodent-Blood-Withdrawal-and-Tail-Biopsy.pdf)

- Place animal in approved animal restrainer.
- Remove any bedding material or feces from the tail. The tail tip must be disinfected with an approved disinfectant (i.e. Betadine)
- Place the animal on a clean work surface.
- Using a fresh scalpel blade, cut 1-2 mm of the distal tail at an angle perpendicular to the work surface (*Figure 38*).
- Apply gentle pressure proximal to the collection site to occlude venous return and ease collection. Collect the blood in a suitable collection device.
- Apply gentle digital pressure to the wound for 30-45 seconds with a clean gauze pad to stop any hemorrhaging. For persistent bleeding, apply a silver nitrate stick, styptic powder or a cautery pen to the wound to stop bleeding.
- Return the animal to its cage only after bleeding has stopped.
- Serial blood samples can be obtained over a short time frame by gently removing the scab without performing an additional cut.
- Only the fleshy portion of the tail tip should be cut. Cutting into the vertebrae is NOT permitted. As only a small portion of the tail does not contain vertebrae, the use of the tail cut procedure should be limited.

*Figure 38: Tail Clip*
5. **Retro Orbital Bleeding**

Retro-orbital or orbital sinus/plexus bleeding (permitted in rats, mice, gerbils, guinea pigs, hamsters) must be proposed to and approved by the IACUC before implementation. The IACUC will permit orbital sinus bleeding when it is scientifically justified, performed with appropriate technique and anesthesia. Veterinary staff experience indicates that this method may lead to orbital damage, blindness and potentially death if not performed correctly. The IACUC encourages the primary use of the submandibular, tail artery or veins; specifically, the nick or cut techniques. These methods are less likely to harm the animal and may be used repeatedly for bleeding. LACs may not train in this technique so training and certification must be obtained from OACU Training and Compliance team or DLAM veterinary services.

Alternating eyes for each bleeding is mandatory, and a week must separate each bleeding. A maximum of two (2) bleedings per eye is permitted. Maximum volume withdrawn within a two-week period is 1.5% body weight. Orbital sinus bleeding requires training and must be performed on anesthetized animals only with IACUC approval.

- On an anesthetized mouse, secure the head between the thumb and the forefinger. The eye should protrude slightly.
- Using a hematocrit tube at the medial canthus of the orbit of the eyeball and gently direct the tube towards the back of the eye socket and rotate it to puncture the sinus and collect the blood (*Figure 40*).
- Following blood collection, hold the eyelids closed to allow the punctured blood vessel to clot and apply ophthalmic ointment to the eye.

*Figure 39: Retro-Orbital Sinus*  
*Figure 40: Retro-orbital Bleed*
6. Cardiac Puncture

This is always a terminal procedure conducted under anesthesia (or shortly after death)!
Cardiac puncture as a method of blood withdrawal permitted in all species provided the following conditions are met:

1. Animal must be under a surgical plane of anesthesia when procedure is conducted.
2. Animal is NOT allowed to recover from anesthesia following the puncture.
3. If the animal is euthanized prior cardiac puncture, training and certification in the technique is not required.
4. A secondary physical method is required to ensure death after blood collection is complete.

**Figure 41a: Cardiac Puncture**

**Figure 41b: Cardiac Puncture**

- Place the animal in dorsal recumbency on a flat, firm surface
- Hold the animal securely by either placing the fingers immediately below the xiphoid process (Figure 41a) or by holding the ribcage between the thumb and the forefinger (Figure 41b).
- Break the seal of the syringe and then insert the needle bevel up, going beneath the ribcage.
- Gently draw back on the syringe and direct the needle towards the heart, remaining vigilant for any blood in the hub of the syringe.
- As soon as blood is visualized, stop moving the needle and gently continue to draw back on the plunger waiting for the syringe to fill.
- Use slow, gentle movements when guiding the needle and drawing back on the plunger. If you are too abrupt in your movements you may lacerate or collapse the heart.

**Figure 42: Cardiac Puncture Diagram**
EUTHANASIA

Performing euthanasia correctly is an ethical imperative. Proper euthanasia is quick, minimizes pain/distress and reliably causes death. Practical issues such as degree of technical difficulty, time required to perform the procedure, readily available equipment/resources to perform the procedure, as well as aesthetics and human emotion must be considered. Sick or injured animals that cannot be successfully treated or relieved of pain and distress should be euthanized promptly. Research personnel are responsible for euthanizing sick, injured or moribund animals as soon as these conditions are noted. Ensure appropriate emergency contact numbers for all research personnel are posted in the animal facility.

Please see the Rodent Euthanasia Policy on the IACUC website for more important considerations for euthanasia as well as information on neonate euthanasia. Please see the Rodent Euthanasia Policy on the IACUC website for more important considerations for euthanasia as well as information on neonate euthanasia. https://research.unc.edu/files/2012/11/Rodent-Euthanasia-Policy.pdf

Euthanasia Methods

There are four primary forms of euthanasia that are practiced on mice at UNC, including 1) CO₂ euthanasia, 2) inhalational anesthesia and 3) injectable anesthesia and 4) unanesthetized physical euthanasia (requires scientific justification). These methods are described in detail below. Note, a secondary physical method is required by the IACUC to ensure death for the first three forms of euthanasia listed. One of the following secondary physical methods must be performed on animals that have been anesthetized with approved agents:

1. Cervical dislocation
2. Decapitation
3. Thoracotomy [open the chest cavity using sharp scissors or scalpel]

Note: The 2013 AVMA Guidelines on Euthanasia recommends that the gradual displacement rate of CO₂ into the euthanasia chamber should be 10-30% to minimize pain and distress. All calculations described below are for a DLAM shoe box style rat cage at 30% displacement.

1. Gaseous Carbon Dioxide (CO₂)

CO₂ must be supplied using a compressed gas tank. The use of dry ice as a source of CO₂ for euthanasia is not permitted. The 2013 AVMA Guidelines on Euthanasia recommends that the gradual displacement rate of CO₂ into the euthanasia chamber should be 10-30% to minimize pain and distress. All calculations described below are for a DLAM shoe box style rat cage at 30% displacement.

Note: DLAM procedure rooms have dedicated CO₂ euthanasia chambers equipped with acceptable flow meters. Investigators who wish to perform CO₂ euthanasia outside of DLAM facilities must adhere to all of the following principals and must purchase the same equipment utilized by DLAM. Appropriate flow meters must be purchased from VWR and can be found through the UNC purchasing system, E-Pro, or at the following website: https://us.vwr.com (part number: 89012-426). To purchase appropriately sized euthanasia chambers, contact DLAM at (919-843-7992).

1. Place the stainless steel lid over the plastic cage. The lid should be connected to a CO₂ tank via a plastic hose.
   a. Make sure the two holes on the top of the lid are not blocked, as these holes allow air to be pushed out by the heavier CO₂.
   b. Make sure the plastic cage does not have an automatic watering opening.
2. Remove each animal from the housing chamber and place into the euthanasia chamber. Never place the housing chamber into the euthanasia chamber. Do not place different animal species in the chamber at the same time. Do not overcrowd the chamber. Each animal should have enough floor space available to lie down.

3. Turn on the valve located on top of the CO2 tank. Next, set the flow meter by adjusting the regulator valve on the left side of the flow meter (see photo on the next page):
   a. Standard DLAM Shoebox style RAT cage: 8 liters per minute (lpm)
   b. Standard DLAM Shoebox style MOUSE cage: 1.8 liters per minute (lpm)
   c. Height x width x length \(=\) liters \(\times\) .20 = flow rate/minute (units = cm)
      i. 61

4. Continue to allow CO2 to flow into the chamber for one minute after breathing stops (approximately 6 minutes for mice and 8 minutes for rats). Young animals, certain strains of mice, and sick animals may require more time to become deeply anesthetized.

5. Once animals are fully anesthetized, immediately perform a physical method of euthanasia (i.e. cervical dislocation, thoracotomy, major organ harvest, or decapitation) to confirm death.

6. Note: If a terminal procedure (i.e. cardiac puncture, tissue collection) must be performed before the secondary physical method, ensure that animals remain deeply anesthetized and that a physical method of euthanasia is performed following the terminal procedure.

7. Place dead animals into a non-PVC containing bag. DLAM provides these bags in a variety of sizes. Label the bag with the ACAP ID#. Seal the bag securely. Place the bag with dead animal(s) into the DLAM carcass freezer available in each animal facility. Please see the Policy on Rodent Carcass Disposal for more information.

8. Disinfect the euthanasia chamber bottom after each use.

*Figure 43: CO2 Flow Meter*
2. **Inhalant Anesthetics (e.g. Isoflurane)**

   This anesthetic agent(s) delivered as a volatile gas to the respiratory tract to induce anesthesia. Isoflurane may be used with a vaporizer machine or in the liquid form via the “drop method.” Personnel should minimize their exposure to these agents as some are considered chemical hazards. These agents should only be used in a chemical fume hood, ducted biosafety cabinet or in a system with an active gas scavenging device.

   Induction chambers for inhalational anesthetics must allow animals appropriate floor space without being too large. Chambers that are too large require increased volumes of the anesthetic agent and may result in slow induction time. Where applicable, induction chambers must prevent animals from coming into direct contact with an anesthetic soaked material. The lid should fit snugly and the chamber must be used in a fume hood, a ducted biosafety cabinet, or with a properly functioning active scavenging system.

   1. Pre-charge the anesthetic chamber by opening the vaporizer or placing two to three pieces of absorbent material on the bottom of the chamber (isoflurane drop method). Add approximately 3-5 mls of isoflurane liquid to the absorbent material (amount of isoflurane is determined by the size of the chamber). Close the lid and wait 5 minutes for the liquid to form a volatile gas within the chamber.

   2. Remove the lid of the chamber, quickly place the animals in the chamber, ensure the absorbent material is not in direct contact with the animal, and immediately close the lid.

   3. The animals should become anesthetized in 2-5 minutes. Neonates require a longer period of time to anesthetize and should remain in the chamber for at least five (5) minutes.

   4. When animals are completely recumbent and obviously deeply anesthetized, remove them from the chamber.

   5. **Immediately** perform a physical method of euthanasia. Isoflurane is highly volatile and animals will quickly regain consciousness once removed from the chamber. Therefore, it is imperative that physical euthanasia be performed immediately.

   6. Note: If a terminal procedure (i.e. cardiac puncture, tissue collection) must be performed before the secondary physical method, ensure that animals remain deeply anesthetized and that a physical method of euthanasia is performed following the terminal procedure.

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*Figure 44: Inhalational Vaporizer*  
*Figure 45: Isoflurane Drop Chamber*
3. Injectable Anesthetics

This is a chemical agent(s) administered by injection with a needle and syringe to induce anesthesia. Common routes of injection include, but are not limited to, intraperitoneal (IP), intramuscular (IM) or intravenous (IV). Injectable anesthetics are easy to administer, require minimal equipment, and avoid safety concerns associated with inhalants.

Injectable anesthetics can be effectively used to anesthetize animals prior to performing physical euthanasia. The agent should be an anesthetic recommended for the species, and the dosage used should be equal to or greater than the standard published reference dose for anesthesia (e.g., a common dose of pentobarbital for euthanasia is 100 mg/kg, which is approximately twice the anesthetic dose for rats and mice). Once the injectable anesthetic is administered, allow sufficient time for the animal to lose consciousness. The drugs that provided the most effective anesthesia are available only to veterinarians and as a result were considered impractical for use by the scientific community. Contact a DLAM veterinarian for more information about appropriate doses of injectable anesthetics.

4. Unanesthetized Physical Euthanasia

Individuals who perform physical euthanasia on unanesthetized animals must first be trained and certified by IACUC approved designees. Physical euthanasia on unanesthetized animals, irrespective of age, can only be done if the procedure is described in the approved ACAP. There are two accepted methods of unanesthesized euthanasia, with requirements for each described below. Note, LAC’s are not allowed to certify others for this technique, training must be done by DLAM veterinary services or the Training and Compliance Team.

- **Cervical Dislocation**

  Cervical dislocation in unanesthetized neonatal and adult rodents is permitted only if it is performed correctly by a trained person, and it is described in an approved ACAP. Manual cervical dislocation is a humane method of euthanasia when limited to rodents weighing less than 200 grams. Personnel using cervical dislocation must be adequately trained, demonstrate their technical proficiency, and must consistently apply this method humanely and effectively.

- **Decapitation**

  Decapitation in unanesthetized neonatal and adult rodents is permitted only if it is performed correctly by a trained person, and it is scientifically justified in an approved ACAP. When performed properly this technique is nearly instantaneous and is considered humane. Guillotines that are designed to accomplish decapitation in adult rodents in a uniformly instantaneous manner are commercially available. Sharp scissors can be used to decapitate neonatal rodents. Check guillotine and scissor blades frequently to ensure sharpness. The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades. The use of plastic cones to restrain animals appears to minimize stress from handling, minimize the chance of injury to personnel, and improves positioning of the animal in the guillotine.

Note: The Physics Department’s Instrument Shop, located in Phillips Hall 115A, will sharpen blades for a small fee (919) 962-1183.
Supply and Vendor Information

Isoflurane can be purchased from the UNC-CH Hospital Pharmacy Storeroom. Call (984) 974-1366 for more information or access an order form online through the Clinic Resources website. Select "Click to Order Online" for University Customers under the Quick Links section to complete the necessary information. A grant number and department number is required for purchase.

**Braintree Scientific**
Phone: 781-843-2202
Website: [www.braintreescience.com](http://www.braintreescience.com)
Description: Instruments, lab equipment, isothermal pads, tattoo paste

**Diamondback Drugs**
Phone: 866-578-4420
Website: [www.diamondbackdrugs.com](http://www.diamondbackdrugs.com)
Description: Veterinary grade pharmaceuticals-including Pentobarbital

**Fisher Scientific**
Phone: 800-766-7000
Website: [www.fishersci.com](http://www.fishersci.com)
Description: Lab equipment, chemicals, instruments, pharmaceuticals

**Henry Schein**
Phone: 800-872-4346
Website: [www.henryschein.com](http://www.henryschein.com)
Description: Veterinary supplies, instruments, pharmaceuticals
*Need Vet License or Researcher DEA license*

**Kent Scientific**
Phone: 888-572-8887
Website: [www.kentscientific.com](http://www.kentscientific.com)
Description: Surgical equipment, telemetry equipment

**Med-Vet International**
Phone: 800-544-7522
Website: [www.shopmedvet.com](http://www.shopmedvet.com)
Description: Veterinary supplies and instruments (discounted)
*Need Vet License*

**National Band and Tag**
Phone: 859-261-2035
Website: [www.nationalband.com](http://www.nationalband.com)
Description: ID tags, ear tags

**Patterson Veterinary**
Phone: 800-225-7911
Website: [www.pattersonvet.com](http://www.pattersonvet.com)
Veterinary supplies, instruments, pharmaceuticals including Pentobarbital
*Need Vet License or Researcher DEA license*

**Plas Labs**
Phone: 800-866-7527
Website: [www.plas-labs.com](http://www.plas-labs.com)
Description: Rodent restrainers, equipment

**Roadrunner Compounding Pharmacy**
Phone: 1-877-518-4589
Contact (as of 2/17): Coy Privette
CoyP@Roadrunnerpharmacy.com

**Roboz**
Phone: 800-424-2984
Website: [www.roboz.com](http://www.roboz.com)
Description: Specialize in instruments

**TW Medical**
Phone: 888-787-4487
Website: [www.twmedical.com](http://www.twmedical.com)
Description: Veterinary supply (Bill Forrester)

**UNC-CH Materials Management and Distribution**
Phone: 966-5671
Description: Scientific Storeroom, General Storeroom, Chemical Storeroom

**Vetamac**
VETERINARY ANESTHESIA SERVICE AND MANUFACTURER
Cynthia A. Burns (as of 2/17), North Carolina Service Technician/Sales Associate
Cynthia.Burns@vetamac.com
Vendors Continued:

**Veterinary Medical Supply**  
Phone: 800-533-8674  
Description: Veterinary Supplies out of Zebulon, NC  
*Need Vet License*

**Southern Anesthesia**  
Phone: 800-456-0757  
Website: [http://www.sasvet.com/](http://www.sasvet.com/)  
Description: This is a human source company that has a Veterinary division, will set up an account without a vet license

**Acknowledgements:**

The University of North Carolina would like to thank:  
The AALAS Learning Library  
Oregon State University  
National Centre for the Replacement, Refinement & Reduction of Animals in Research  
Jackson Laboratories  
University of Minnesota, Research Animal Resources  
McGill University  
University of Texas Medical Branch at Galveston

**References**

**ASK YOUR PI or LAC WHAT TECHNIQUES YOU NEED BEFORE CLASS!**

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<td>*Provided during a 1-on-1 training session. Please call the OACU to schedule an appointment 966-5569</td>
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<td>Inhalational Anesthesia Vaporizer Machine</td>
<td>Contact DLAM Vet Services at 966-2906</td>
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**Proficiency Rating**

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I certify that I have received the above training:

Signature: __________________________
Print Name: __________________________
PID: __________________________
PI: __________________________
Instructor Signature: __________________________
Date: __________________________