Basic Rat Handling and Technique Workshop
UNC-IACUC

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

Basic Information about working with Rats

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 RAT 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 (see techniques sheet on page 28 for details).

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/](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/](http://research.unc.edu/offices/nlac/)
Rat Behavior

1. Rats are basically docile, curious animals that respond to quiet, gentle handling. They are not usually aggressive (except for some strains), but can inflict painful bites if frightened.

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

3. Rats are creatures of habit. Every day events do not tend to stress or excite the rats. However, handling and restraint can be stressful and result in the rat being difficult to work with. Conditioning the rat to such handling (so they do not associate handling and restraint aversively) can make the animals much easier to work with. This reduces stress for both the animal and the handler.

4. Healthy rats have a groomed appearance, with bright, alert eyes and a smooth hair coat (Figure 1). Unhealthy or rats demonstrating signs of illness or pain may be less alert, have a ruffled or unkempt hair coat and a hunched posture (Figure 2).

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

Figure 1: Healthy Rat

Figure 2: Unhealthy Rat

Figure 3: Malocclusion

Figure 4: Malocclusion Scissors
Sex Determination

Gender in rats is determined by comparing anogenital distance, or the distance between the urogenital opening and the anus. Males typically have a larger anogenital distance when compared with the females.

*Figure 5: Adult Male and Female Rat*

![Adult male rat (L). Note greater anogenital distance marked by black line](image1)
![Adult female rat (R). Note shorter anogenital distance and vulva marked by black line](image2)
Handling and Restraint

Body Scoop
This form of restraint is ONLY good for docile rats.
   a) Support abdomen with cup of hand.
   b) With firm, but gentle pressure, grasp the rat around the thorax with the thumb and index finger under each of the front legs.

Tail hold
For aggressive rats or rats you are not familiar with.
   a) Grasp the animal at the base of the tail and lift.
   b) Support the body weight at all times, do not dangle by the tail. This hold is safe for short periods of the time, and care must be taken not to damage the tail as it is easily broken and/or stripped of skin.
   c) Place on arm of lab coat (This helps them to relax as they become tense on smooth surfaces).

Restraint
Take care how much pressure is placed on the chest cavity. You do not want to interfere with respiration. Look for gasping, blue color, bulging eyes or blood from the nose. Gloves, either light leather, cloth or mesh greatly diminish sensitivity, increasing chance of injury to rodents. They also make it more difficult to perform delicate procedures. Rats can often bite through gloves.

Scruffing
- It has been our experience that most rats resent being scruffed. They do not have as much loose skin in and around the shoulder as mice and hamsters.

Restrainers
- Should be easy to disinfect, easy to place the animal in, safe for both the animal and the handler.
- There are many commercially available restrainers for rodents
Hand Restraint

1. **Basic/Four finger hold.** Lay hand on rat’s back, place head between index and middle finger, as close to the base of the fingers as possible. Use thumb and last 2 fingers along body to support chest. Grasp tail base with other hand and support body (*Figures 10 & 11*).

2. **Shoulder/Two finger hold.** Grasp the tail and gently pull the rat backwards. With the other hand slide thumb and index finger up under shoulders forcing them upwards until the forelegs are crossed. Backward tension must be maintained on the tail, gently stretching the animal to maintain upward lift of the shoulders. In this position the rat is unable to bite the handler (*Figure 12*).

3. **Combination/Three finger hold.** Grasp the tail and while exerting gentle backward tension on the tail, slide the other hand forward, placing the thumb under the leg and shoulder on one side, and the index finger on top of the shoulder and alongside the neck on the other side. The middle finger is placed under the foreleg with the index finger sitting above. This hold is only applicable for techniques such as ear tag/notch, where the rat can remain on the work surface. (*Figure 13)*.

4. **Towel roll.** Place the rat on a hand towel or surgical towel. Drape one end of the towel over the rat and gently roll the rat in the towel securely. Place the rat’s head and upper body under your arm while supporting the lower body with the same forearm or hand. Rats usually feel calm and secure with this hold.

---

**Figure 10: Four Finger Hold**
*Diagram of Finger Placement*

**Figure 11: Four Finger Hold**
*(Numbers correspond with Figure 10)*

**Figure 12: Two Finger Hold**
*(Recumbent Rat)*

**Figure 13: Combination/Three Finger Hold**
An Alternative Method of Rat Restraint

By Lori Roberts, LVT and Melissa Dyeon, DVM, Unit for Laboratory Animal Medicine Training Core, University of Michigan, Ann Arbor, MI

Creating comfortable restraint for animals that facilitates efficient accomplishment of procedures while minimizing stress is integral to the success of a research project. Reducing time and manpower are also concerns for research technicians and investigators alike. A novel approach to rat restraint has been employed by our staff for such tasks.

A square, washcloth-sized cloth is folded into a triangular shape. The folded cloth is sewn together for approximately half of the length of one of the open sides, leaving a small opening at the tip for air flow to restrained animals. The sewn cloth forms a cone shape (Fig. 1).

Place the rat's head into the closed end of the restraint device (Fig. 2). Fold the end of the cloth over the rat's back and hind quarters (Fig. 3). While maintaining the rat's position by gently pushing forward with a stabilizing hand, carefully place a binder clip over the posterior end of folded cloth, being careful not to catch the rat's skin (Fig. 4). Place a second binder clip on the anterior portion of the folded cloth (Fig. 5).

The rat is easily and gently restrained in the soft cloth, leaving the technician with both hands free (Fig. 6). Respiratory rate can be easily monitored by movement of the chest wall. The limbs can be removed from slits in the cloth for manipulation (Fig. 7). The tail is free to be accessed for injections or blood draws (Fig. 8). The tip of the "cone" can be cut to various sizes to fit different sizes of rats so the animals can breathe freely (Fig. 9). The color of the skin and mucous membranes can be assessed through the opening to monitor the animal.

The restraint device is opaque, soft, and gently molds to the animal to provide secure restraint. Most rats quickly become trained and enter the restraint readily. Most rats are also very calm while inside the restraint device.

Regular application of this technique by our staff has helped to decrease the amount of time and manpower needed to perform injection and blood collection on large numbers of animals. Less experienced personnel are able to learn the restraint technique very quickly. Personnel report that they feel more comfortable completing required tasks while the rat is held safely in restraint that allows the technician to have both hands free.

The cloth is both inexpensive and sanitizable. Many types of material can be used to construct the restraint device, based on the needs and resources of the users. We have used both terrycloth and chamois successfully. Additionally, varied sizes of cloth can be used to properly restrain different sizes of rats.
Injections
Basics and a few tips

- Use a fresh, sterile needle for each injection
- To avoid excessive leaking, do not remove the needle from the animal for a few seconds following injection
- Always inject with the bevel of the needle facing up to reduce skin trauma
- Do not reuse needles between animals
- When giving substances intravenously, inject slowly to avoid shock. Clear air from needle before injecting.
- Injecting slowly when giving substances intramuscularly will cause the least amount of pain.

*Figure 14: Parts of the Syringe and Needle*

![Image of syringe and needle parts]

*Figure 15: Skin Layers*

![Image of skin layers]

**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 ~ 300 g rat)</th>
<th>Aspiration Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraperitoneal (IP)</td>
<td>23-25G</td>
<td>20 ml/kg</td>
<td>20 ml/kg (6.0 ml)</td>
<td>No</td>
</tr>
<tr>
<td>Subcutaneous (SQ, SC)</td>
<td>25G</td>
<td>5.0-10 ml/kg</td>
<td>10 ml/kg/site (3.0 ml)</td>
<td>Yes; inject if no blood in the needle hub</td>
</tr>
<tr>
<td>Intramuscular (IM)</td>
<td>25G</td>
<td>0.1 ml/site</td>
<td>0.2 ml/site (20 μl)</td>
<td>Yes; inject slowly if no blood in the needle hub</td>
</tr>
<tr>
<td>Intravenous (IV)</td>
<td>21-23G</td>
<td>2.0-4.0 ml/kg/hr</td>
<td>4.0 ml/kg/hr (1.2 ml)</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.
   - Hold the rat in dorsal recumbency with the tail firmly restrained (under your wrist or by another person). Two people may be required to safely perform this procedure in large rats (*Figure 16*).
   - Insert needle; bevel up in a position midway between the bend of the knee and the midline. This position allows for safe injection avoiding the bladder and other organs such as the liver or spleen (*Figure 17*).

   ![Figure 16: IP Injection Restraint](image1)
   ![Figure 17: Target IP Injection Site](image2)

2. **Subcutaneous injections**
   This injection is administered beneath the skin and above the underlying muscle. It may be performed in any area of loose skin along the back or flank.
   - Scruff the rat to tent the skin, while pressing the head towards the surface for maximum restraint, and insert the needle, bevel up, in the pocket created.
   - Aspirate. If no blood appears in the hub of the needle, inject slowly (*Figure 18*).
   - Large amounts may be administered, but take care to avoid distending the skin.

   ![Figure 18a: Subcutaneous Injection](image3)
   ![Figure 18b: Subcutaneous Injection](image4)
3. **Intramuscular injections**
   This injection is made into the posterior thigh of the rat. 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 21).
   - Hold the rat against your body and restrain the leg with one hand (Figure 19). Alternatively, a restrainer may be used (Figure 22).
   - Insert the needle angled parallel to the femur and aspirate (Figures 19 & 20). 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 19: Intramuscular Injection Restraint  
Figure 20: Intramuscular Injection  
Figure 21: Intramuscular Injection Site  
Figure 22: Alternate Restraint Method*
4. **Intravenous injections**

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

- Place the rat into a restrainer and dilate the blood vessels by warming them if needed. Warm water, a heat lamp, or hand warmers may be used for this purpose.
- Locate the dorsal vein. When the rat 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 24*). 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 23 & 25*).
- The vein will cleared 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. Another indicator of a successful injection, when clearing cannot be visualized, is plunger pressure. If it is difficult to depress the plunger once in the tail, the needle is likely subcutaneous. It should be easy to press the plunger if the needle is properly placed in the vein.
- 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 towards the base of the tail the vein is located more deeply.

*Figure 23: Cross Section of Rat Tail*

*Figure 24: Lateral Tail Veins*

10 o'clock & 2 o’clock position

*Figure 25: Intravenous Injection*
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 insure that you insert the needle to the proper depth. If the tube is too short, the injected fluid may be aspirated by the rat 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 rat 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 26*).
- 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 rat’s head back, so that the neck is in a straight line (*Figure 27*).
- The needle should pass easily down the esophagus; with little to no resistance. If the rat struggles or resistance is met, stop, back up and start over.
- Observe the rat carefully after the gavage is completed. No fluid should be coming from the mouth or nose and the rat should not show signs of distress.

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

<table>
<thead>
<tr>
<th>Rat Wt (g)</th>
<th>Gauge</th>
<th>Length (inches)</th>
<th>Ball Diameter (mm)</th>
<th>Oral Gavage Volume per weight (suggested)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50-75 g</td>
<td>20</td>
<td>1” – 3”</td>
<td>1.9 - 2.25</td>
<td>30g: 1mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50g: 2mL</td>
</tr>
<tr>
<td>75-120 g</td>
<td>18</td>
<td>1” - 1.5”</td>
<td>2.0 - 2.25</td>
<td>100g: 3mL</td>
</tr>
<tr>
<td>100-200g</td>
<td>18</td>
<td>2” – 3.1”</td>
<td>2.0 - 2.3</td>
<td>200g: 4mL</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>1” – 3.3”</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>2” – 3”</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>150-300g</td>
<td>15 - 16</td>
<td>3” – 4”</td>
<td>2.8 – 3.0</td>
<td>300g: 5mL</td>
</tr>
<tr>
<td>200-350g</td>
<td>13 - 14</td>
<td>3” – 5”</td>
<td>2.9 – 4.0</td>
<td>300g: 5mL</td>
</tr>
</tbody>
</table>

Source for Gavage Needle Sizing: Braintree Scientific*
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, opthalmic 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.

Figure 28: Ophthalmic Ointment

Figure 29: Check for Toe Pinch Reflex
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 30: 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></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Expiration date</th>
<th>Schedule: I II III IV V</th>
<th>Highlighted areas required for controlled drug use</th>
<th>Initials are for controlled drug use and waste</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Date</th>
<th>Protocol # &amp; Species</th>
<th>Animal ID/ Group ID</th>
<th># in group</th>
<th>Total Volume injected (mg)</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>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</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

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 31). 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.

   Figure 31: Ear Notch Tools

   - Firmly restrain the rat’s head 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 32 & 33).
   - Firmly and quickly press down and punch the ear, maintaining a firm restraint on the animal to avoid being bitten or tearing of the ear.

   Figure 32: Ear Notch – Short Tool
   Figure 33: Ear Notch – Long Tool
2. Ear Tag

Ear tags can be purchased with numbers and/or letters. Correct placement of the tag makes them fairly 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 rat may experience itching and irritation, which can lead to infection. If the tag is not fully closed, the rat 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 37 and 38 on the following page 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 34).
- Firmly restrain the rat in your non-dominant hand.
- Position the ear tag into the desired position. Aim for the outer ear avoiding the vasculature and also towards the lower half of the ear so that the tag hangs appropriately (Figure 35).
- Firmly close the ear tagging tool until the tag is closed (Figure 36).

*Figure 34: Correct Ear Tag Placement*

*Figure 35: Open Ear Tag*

*Figure 36: Closed Ear Tag*
Figure 37: Open Ear Tag

Figure 38: Closed 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 rats. The chosen route of blood collection should minimize pain and distress to the animal as much as possible while still achieving study objectives. Please see the following UNC Guidelines for Rodent Blood Withdrawal for more details on the techniques described below: http://research.unc.edu/files/2012/11/Guidelines-for-Rodent-Blood-Withdrawal-and-Tail-Biopsy.pdf

\* 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 μl micro capillary tubes (200 μl), may be withdrawn].

1. 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 rat in a restrainer or have another person firmly restrain the animal.
   • Start midway up the tail and nick the artery or vein with a needle or lancet (Figure 39).
   • 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 39: Tail Nick*
2. **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:


- 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 40*).
- 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 40: Tail Clip*

*Figure 41: Tail Clip – Close Up*
3. **Tail Artery Bleed**

Blood may be withdrawn from the ventral tail artery using a plungerless syringe and a 22-gauge (or smaller) needle.

- It may be necessary to anesthetize the animal to facilitate this withdrawal technique, as the artery is located on the ventral surface of the tail, and access is easiest when the animal is laying on its back.
- Warm the tail to help in visualizing the tail artery. A tourniquet placed at the base of the tail may facilitate bleeding.
- Visualize the ventral tail artery and insert a 22 gauge plungerless needle (*Figure 42*).
- Let the blood drip into the collection vessel.

*Figure 42: Tail Artery Bleed*

4. **Saphenous Vein**

This method of obtaining blood is often used when a series of small samples is required.

- Place the rat in a conical tube and shave the caudal surface of the thigh. The saphenous vein can be seen in this area (*Figure 43*). Alternatively, restrain the rat by hand or with anesthesia.
- Apply a lubricant to prevent wicking. Place a tourniquet above the knee or apply firm pressure with your finger, and enter the vein with a 25 gauge needle.
- Micro-hematocrit and microvette tubes may be used to collect the blood (*Figure 44*).

*Figure 43: Saphenous Vein*  
*Figure 44: Saphenous Blood Collection*
5. **Retro Orbital Bleeding**

NOTE: 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 rat, secure the head between the thumb and the forefinger. The eye should protrude slightly (*Figure 45*).
- 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 46*).
- Following blood collection, hold the eyelids closed to allow the punctured blood vessel to clot and apply ophthalmic ointment to the eye.

*Figure 45: Finger Placement for Retro-Orbital Bleed*

*Figure 46: Retro-orbital Bleed*
6. Cardiac Puncture

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

1. Animal is 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 47: Cardiac Puncture - Entry**

**Figure 48: 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 or by holding the ribcage between the thumb and the forefinger.
- Break the seal of the syringe and then insert the needle bevel up, going beneath the ribcage (*Figure 47*).
- Gently draw back on the syringe and direct the needle towards the heart, remaining vigilant for any blood in the hub of the syringe (*Figure 48*).
- 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 49: Cardiac Puncture Diagram**

- 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 or by holding the ribcage between the thumb and the forefinger.
- Break the seal of the syringe and then insert the needle bevel up, going beneath the ribcage (*Figure 47*).
- Gently draw back on the syringe and direct the needle towards the heart, remaining vigilant for any blood in the hub of the syringe (*Figure 48*).
- 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.
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.

Please see the Rodent Euthanasia Policy on the IACUC website for more important considerations for euthanasia as well as information on neonate euthanasia.

Euthanasia Methods

There are four primary forms of euthanasia that are practiced on rats at UNC, including 1) CO$_2$ euthanasia, 2) inhalational anesthesia overdose and 3) injectable anesthesia overdose 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 (only in rats less than 200 grams!)
2. Decapitation
3. Thoracotomy [open the chest cavity using sharp scissors or scalpel]

Note: In addition to DLAM personnel, only research personnel who have been properly trained and are listed on the approved ACAP, can perform these physical methods.

1. **Gaseous Carbon Dioxide** (CO$_2$)

CO$_2$ must be supplied using a compressed gas tank. The use of dry ice as a source of CO$_2$ for euthanasia is not permitted. The 2013 AVMA Guidelines on Euthanasia recommends that the gradual displacement rate of CO$_2$ 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 20% displacement.

Note: DLAM procedure rooms have dedicated CO$_2$ euthanasia chambers equipped with acceptable flow meters. Investigators who wish to perform CO$_2$ 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 CO$_2$ 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 CO$_2$.
      b. Make sure the plastic cage does not have an automatic watering opening.
      c. Invert the cage before placing animals in, to remove residual CO$_2$ that may be left from previous users.

   2. Remove each animal from the home cage and place into the euthanasia chamber. Never place the home cage 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 x .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 are no longer breathing 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 protocol #. 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. [Link](http://research.unc.edu/files/2012/11/Disposal-of-Rodents-Carcasses-Policy.pdf)

8. Disinfect the euthanasia chamber bottom after each use.

*Figure 50: CO$_2$ 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.

---

**Figure 51: Inhalational Vaporizer**  
**Figure 52: Isoflurane Drop Chamber**
3. Injectable Anesthetics

This technique involves 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. All aspects of anesthesia must be approved in the protocol (or in emergencies, recommended by a DLAM veterinarian or IACUC staff) prior to use.

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. Contact a DLAM veterinarian for more information about appropriate doses of injectable anesthetics.

4. 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 unanesthetized 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 IACUC 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 919-966-1366 for more information or go to Ground Floor, room NG10B. A grant number and department number is required for purchase.

**Braintree Scientific**
Phone: 781-843-2202
Website: [www.braintreesci.com](http://www.braintreesci.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: 800-544-752
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
"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/
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!**

<table>
<thead>
<tr>
<th>Technique Needed?</th>
<th>Technique</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Handling</td>
<td></td>
</tr>
<tr>
<td></td>
<td>and</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Restraint</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intraperitoneal Injection</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Subcutaneous Injection</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oral Gavage</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ear Notch (punch)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ear Tag</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intramuscular Injection</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intravenous Injection</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tail Nick Bleed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tail Clip Bleed/Genotyping</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anesthesia – Injectables</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tail Artery Bleed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CO₂ Flow Meter w/ Phys. Euthanasia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cardiac Puncture, <strong>terminal</strong></td>
<td></td>
</tr>
</tbody>
</table>
|                   | Retro-orbital Bleed | "individual appointment during class"*  
|                   | | "training provided only if included within animal protocol"** |
|                   | Retro-orbital Injection | "individual appointment during class"*  
|                   | | "training provided only if included within animal protocol"** |
|                   | Cervical Dislocation Without Anesthesia (<200g) | "individual appointment during class/ one on one session"*  
|                   | | "training provided only if included within animal protocol"** |
|                   | Decapitation Without Anesthesia | "individual appointment during class/ one on one session"*  
|                   | | "training provided only if included within animal protocol"** |
|                   | Isoflurane **Euthanasia / Anesthesia Drop Method** | "provided during a 1-on-1 training session. Please call the OACU to schedule an appointment: 966-5569" |
|                   | Inhalational Anesthesia Vaporizer Machine | Contact DLAM Vet Services at 966-2906 |

**Proficiency Rating**

I certify that I have received the above training:

Signature: ________________________________
PID: ________________
Print Name: ________________________________
PI: ________________
Instructor Signature: ________________________
Date: ________________

UNC Chapel Hill   Revised 2/2017