UNC-Division of Comparative Medicine (DCM)

Basic MOUSE Handling and Technique Guide

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

Basic Information about Working with Mice

Proper Personal Protective Equipment (PPE) is a requirement for working with animals. In DCM facilities, minimum requirements include a disposable gown 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.

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 Division of Comparative Medicine (DCM) and demonstrate proficiency before training others within their lab. Please note, there are certain techniques that LACs are not allowed to train in.
2. For additional training please contact the DCM Training Team via the IACUC office at 966-5569 or iacuc@med.unc.edu. We offer training in both one on one and classroom settings.

For a look at IACUC Guidelines and training information, visit our websites:
https://research.unc.edu/iacuc/training/ **OR** https://research.unc.edu/comparative-medicine/training/

To register for classes, click this link, and search under 'Office of Animal Care and Use':
https://apps.research.unc.edu/events/index.cfm

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 Comparative 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. While not always aggressive, they 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 *(Figures 2a & 2b).*
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. 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.

Figure 1: Healthy Mouse

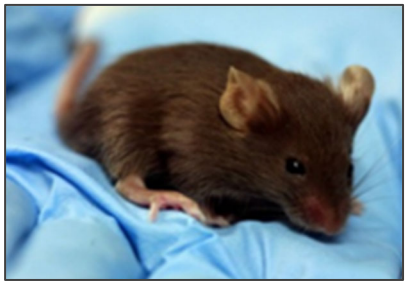


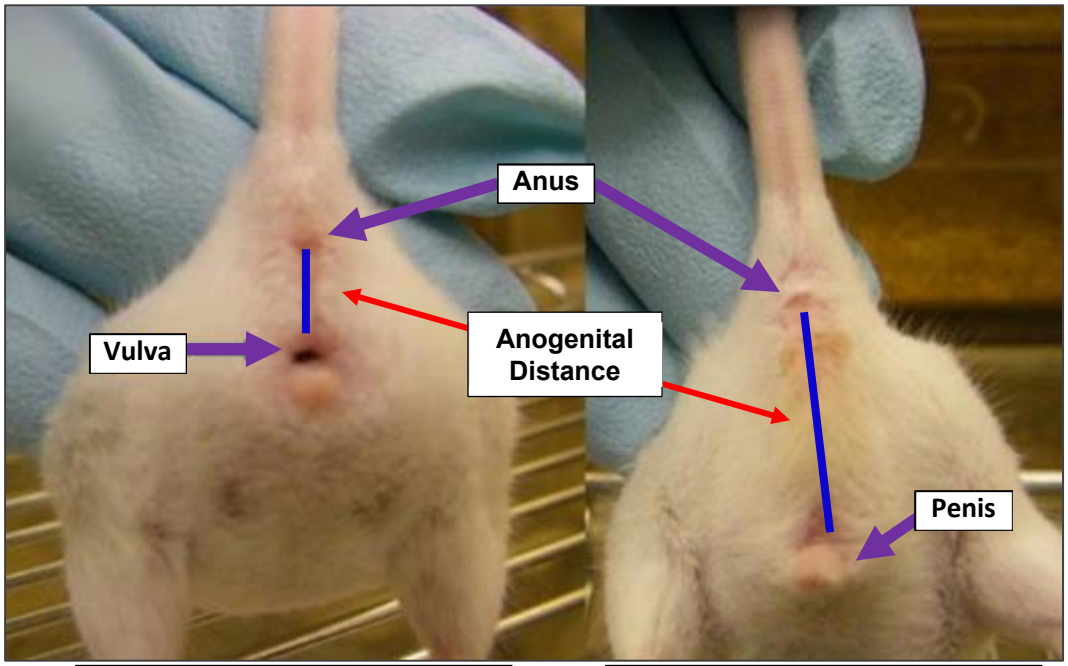
Figure 2a: Unhealthy Mouse

Figure 2b: Unhealthy Mouse

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 3 and 4* below.

Figure 3: Sex Determination in Adult Mice



Adult female mouse. Note shorter anogenital distance marked by the blue line.

Adult male mouse. Note longer anogenital distance marked by the blue line.

Figure 4: Sex Determination in Young Mice

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 5a*). It is best that the surface is not slick or smooth as mice will behave much more calmly if they have firm footing. Keeping hold of the tail, place the thumb & first finger of the opposite hand on the lower back. Gently press down & forward until you reach the extra skin at the back of the neck, touching the ears. (*Figure 5b*). Firmly pinch the loose skin straight up (*Figure 5c*), lift the mouse, tug the tail towards your wrist and secure the tail with ring/pinky finger *(Figure 5d)*. The tail can then be held by the last two fingers as shown (*Figure 5e*). Your grip should be firm enough to keep the mouse from struggling, but gentle enough for it to breathe comfortably.

Figure 5a

Figure 5c

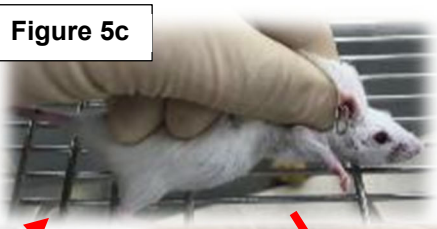


Figure 5e

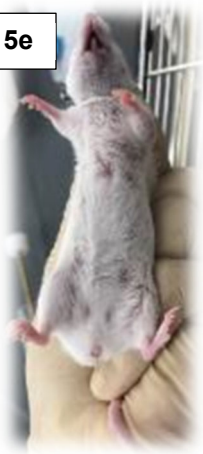


Figure 5b

Figure 5d

Figure 6: Three finger hold

Another restraint method that can be used is the three-finger hold *(Figure 6)*. Use steps from *Figures 5a/b* to begin your restraint technique, then use your index and middle fingers to initially pinch the skin. Once you have a good scruff you can replace your middle finger with your index finger and continue to lift the mouse and secure the tail with your ring/pinky finger *(Figures 5d & e)*.

For quick handling, such as cage to cage transfers, it is acceptable to use forceps (*Figure 7*). 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 an approved disinfectant (e.g., Peroxigard) between cages.

Another method of transferring mice is referred to as the "tube" or tunnel method *(Figures 8a & 8b)*. 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.

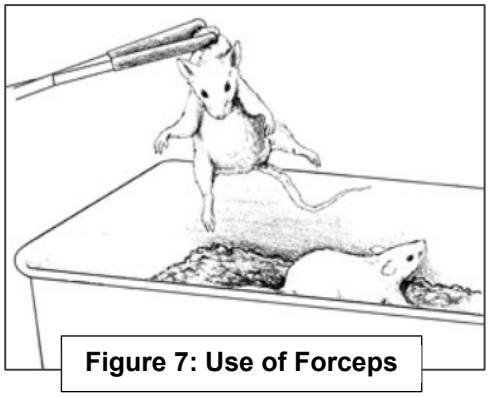


Figure 7: Use of Forceps

Figures 8 a & b: Tube Method

Examples of Commercial Restrainers:

Various styles and sizes available: See "Supply and Vendor Information" for more details.

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.

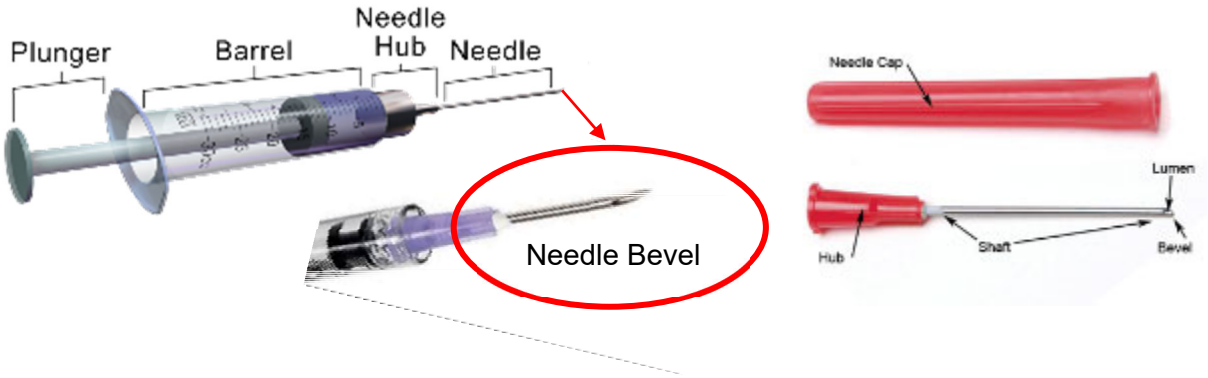


Table 1: Recommended Volume and Needle Gauge by Injection Type

Injection Type	Ideal Needle Size (Gauge)	Recommended Volume**	Maximum Volume (in ~ 25 g mouse)	Aspiration Required
Intraperitoneal (IP)	25-27G	20 ml/kg	40 ml/kg (1ml)	Yes
Subcutaneous (SQ, SC)	25-30G	10 ml/kg	40 ml/kg (1ml)	Yes, inject if no blood in the needle hub
Intramuscular (IM)	27-30G	0.025 ml/site (25µL)	0.05 ml total (50µl)	Yes, inject slowly if no blood in the needle hub
Intravenous (IV) (*continuous)*	25-28G	2.0-4.0 ml/kg/hr	4.0 ml/kg/hr (100µL)	No; inject slowly
Intravenous (IV) (*bolus*)	25-28G	5.0 ml/kg	5.0 ml/kg (125µl)	No

**Adapted from "Formulary for Laboratory Animals, 3rd, Ed. Hawk, Leary and Morris, 2005.*
***Source for Volume: University Of North Carolina at Chapel Hill Standard for Administered Agents*

1. Intraperitoneal Injections

This injection is made into the lower 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 9).*
- Insert the needle bevel up in a position below the bend of the knees; to the left or right of the midline *(Figure 10).*
- Angle the needle approximately 30°-45° to the body, aspirate – if no blood is seen in the needle hub, inject your medium *(Figure 9).*

Figure 9: IP Injection Restraint

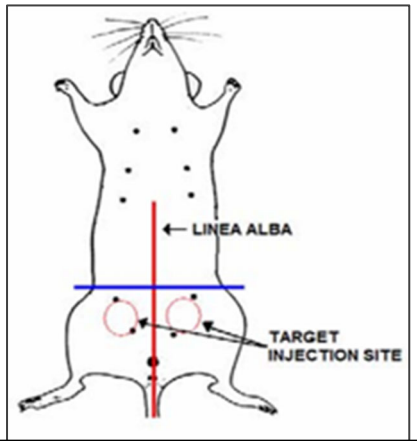
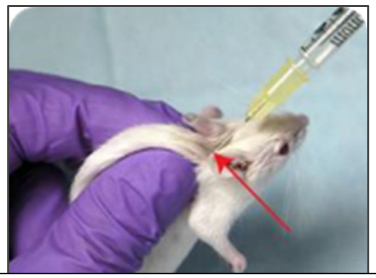


Figure 10: Target IP Injection Site

2. Subcutaneous Injections

This injection is administered beneath the skin and above the underlying muscle (Figure 12). It may be performed in any area of loose skin along the back or flank.

- Firmly scruff the mouse and create a pocket of loose skin or 'tent' between the shoulder blades, over the flank, or anywhere there is loose skin *(Figure 11).*
- 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.\



Figures 11 a & b: SQ Injection

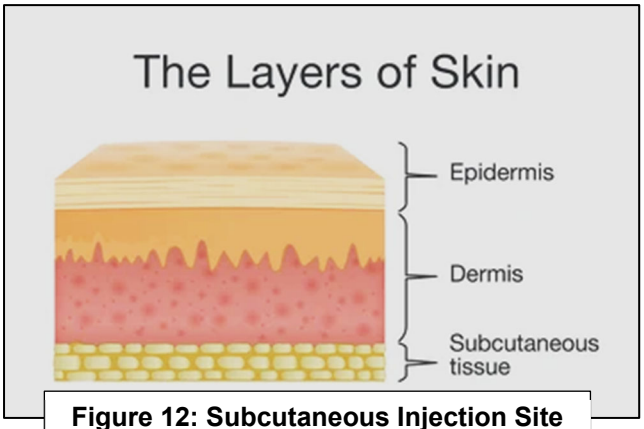


Figure 12: Subcutaneous Injection Site

3. Intramuscular injections

This injection is made into the muscle mass of 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 13).*
- 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 14).*
- If not using restrainer, perform normal restraint and rotate mouse to expose rear leg *(Fig. 15)*

- <u>If using a restrainer</u>, 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 *(Figure 16a).*
- Insert the needle parallel to the femur and perpendicular to fur growth, at a shallow angle. Aspirate to ensure the needle is not in a blood vessel, then proceed with the injection if no blood is noted in the hub. *(Figures 14, 15 & 16b).* If blood is seen, remove and re-insert the needle and try again.
- Care must be taken to avoid the sciatic nerve and the femoral vein, artery and nerve.

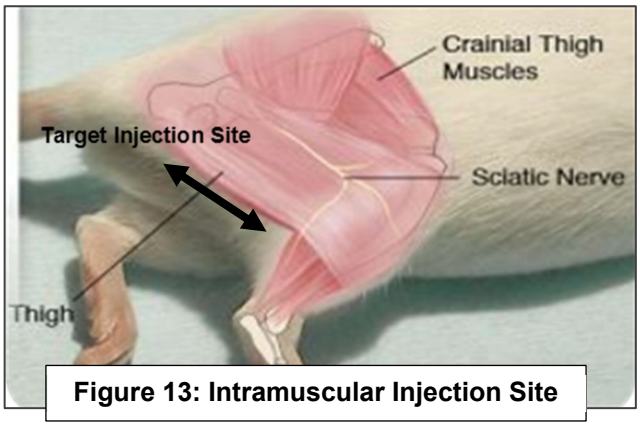


Figure 13: Intramuscular Injection Site

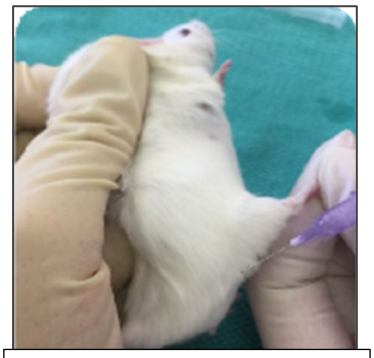
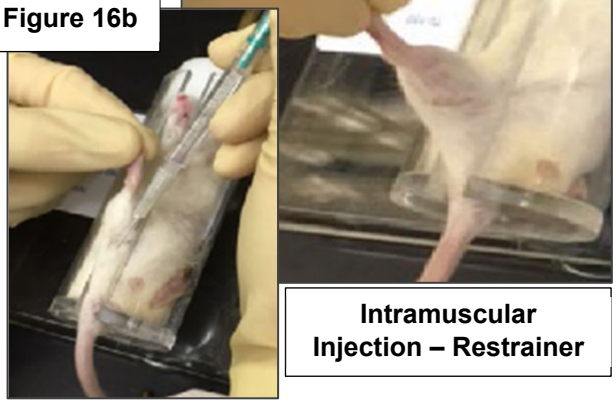


Figure 14: Intramuscular Injection – 2 people

Figure 15: Intramuscular Injection – 1 person

Figure 16a

Figure 16b



Intramuscular Injection – Restrainer

4. Intravenous injections

This injection is done into one of 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 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, then locate the left and right lateral tail veins, in the ~10 and 2 o'clock positions *(Figure 18)*. Injections can be made into either of these veins.
  - Warm water, a heat lamp, or hand warmers *may* be used to dilate the blood vessels.

- Start as far away from the base of the tail as possible with the needle positioned horizontally to the vein. Insert the needle bevel up just until the bevel is under the skin. Needle should be almost parallel to the orientation of the tail and inserted shallowly to prevent passing through the vein. *(see Figure 17-19 below).*
- Depressing the plunger should be as easy as pushing it in open-air. The vein may 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.

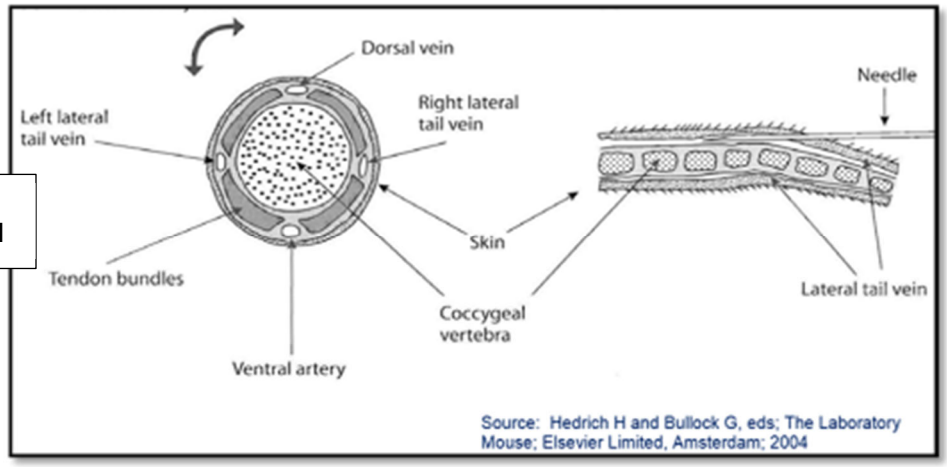


Figure 17: Cross Section of Mouse Tail

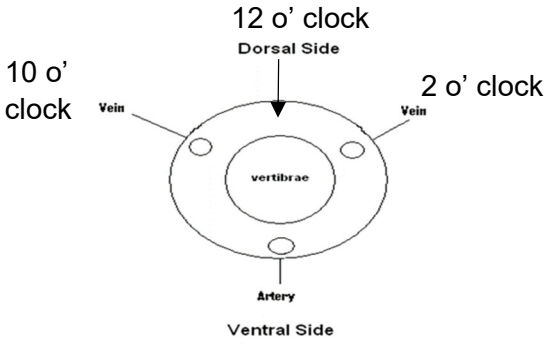


Figure 18: Lateral Tail Veins at 10 and 2 o'clock

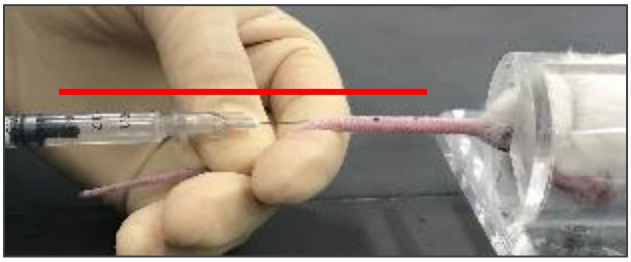


Figure 19: Tail Vein Injection

5. Oral Gavage

A blunt, ball-tipped gavage needle is used to deliver substances directly to the stomach (Figure 20) bypassing swallowing. 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.

- Firmly restrain the mouse to measure the needle before inserting. The needle should measure the distance from the corner of the mouth to the xiphoid process (near last rib) *(Figure 21)*.
- Insert the gavage needle into one side of the mouth at about a 45° angle. When the needle-tip hits the roof of the mouth, 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 *(Figures 22 a & b)*.
- The needle should pass easily down the esophagus with little to no resistance. If resistance is met, gently twist the needle and pause to allow the mouse to swallow. If the mouse struggles or the needle will still not pass any further, 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***

Mouse Wt (g)	Gauge	Length (inches)	Ball Diameter (mm)	Oral Gavage Volume per weight* *Recommended: ≤ 5 ml/kg*
Up to 14g	24	1	1.25	70µl (14g mouse)
15-20g	22	1 or 1.5	1.25	75-100µl
20-25g	20	1, 1.5 or 3	2.25	100-125µl
25-30g	18	1, 1.5 or 2	2.25	125-150µl
30-35g	18	2 or 3	2.25	150-175µl

** Source for Volume: University Of North Carolina at Chapel Hill Standard for Administered Agents*
****Source for recommended sizing: Braintree Scientific*

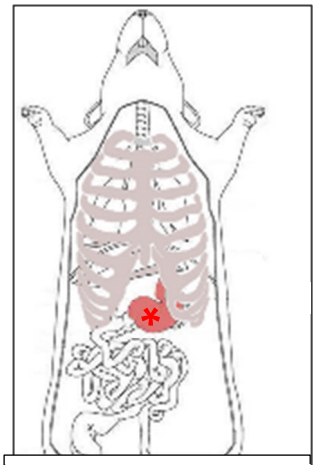


Figure 20: Location of Stomach

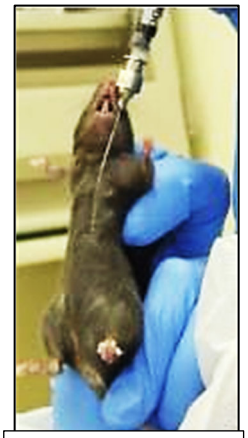
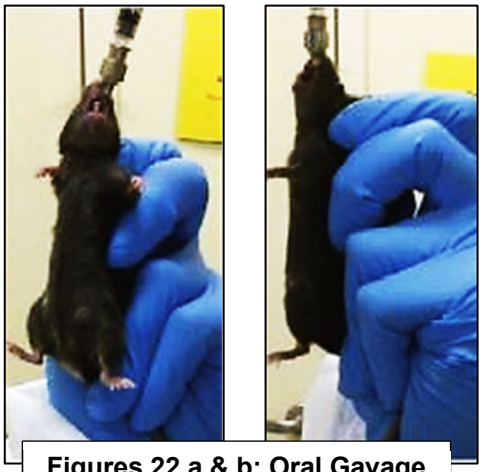


Figure 21: Measurement



Figures 22 a & b: Oral Gavage

Anesthesia Injectables

Anesthesia injectables may be utilized for survival surgeries. Please see the IACUC Anesthetized Procedure Standards for Rats and Mice for more details about IACUC expectations for surgical procedures: Anesthetized Procedure Standards for Rats and Mice (Non-survival and Survival Procedures)

- Weigh the animal to calculate the appropriate drug dosage.
- Administer IACUC protocol-approved anesthetic drug(s) based on weight and correct dose, via the approved injection route.
- Place animal into a clean, empty cage with no bedding, no other animals, and on a heat source.
- Animal (including tail) should stay on the appropriate heat source from initial drug administration through the procedure, until the animal is fully awake and recovered post-surgery.

- Care should be taken when using heat sources, to avoid overheating and burns.
- Monitor animal during induction (initiation of anesthesia) until it is in recumbency in the cage (lying down).
- Pick up animal and check for a blink reflex. When the blink reflex is absent, a pharmaceutical or veterinary grade ophthalmic ointment must be applied with a secondary applicator to retain moisture *(Figure 23)*. Failure to apply the ointment may result in irritation, infection, and corneal ulcers.
- Check for pain response by performing a deep toe pinch on all 4 feet using a firm fingernail to fingertip pinch directly on the toe joints *(Figure 24)*. No response from all 4 feet is required prior to performing painful procedures.
    - Response = visible or tactile muscle movement, change in breathing pattern, etc.
- Anesthetized animals must be observed at all times during anesthesia (including induction and recovery) and must not be left unattended until sternal and ambulatory (able to right itself and walk around normally).
- Return animal to housing room only when fully awake and ambulatory.

Figure 23: Ophthalmic Ointment

Figure 24: Deep Toe Pinch

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 DCM 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 on the controlled drug log per DEA requirements (Figure 25).*

Figure 25: Example of Drug Log Template

Drug Name _____ Controlled substance Yes / No **Concentration, dose & route**

(for mixture, give the amounts under concentration/dose) (for mixture, include dose of each)

Initial (start) volume _____ Schedule: I II III IV V _____

Expiration date _____

- Highlighted areas required for controlled drugs
- Initials are for controlled drug use and waste

Date	Protocol # & Species	Animal ID/ Group ID	# in group	Total Volume Injected (ml)	Amount Waste (ml)	Amount Left in Bottle	Initials	Tac Pinch	Procedure & Notes

Date Implemented: 9/2015 [reviewed and endorsed by DLAM Veterinarians] Revised 6/2016

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**: Standard on Rodent Identification*

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.

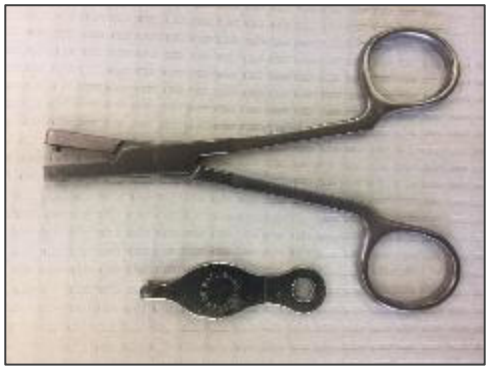


Figure 26: Ear Notch/Punch Tools

- Firmly scruff the mouse. The head should be immobile to avoid tearing the ear tissue.
- Place the flat side of the ear notching tool on the ear so that you can see the area you will be punching.
- Notch the outer edge of the ear to avoid the vasculature and excessive bleeding *(Figures 27 a & b)*.
- Firmly and quickly press down to punch the ear. Collect the tissue for genotyping if needed.

Figures 27a: Ear Notch/Punch with Short Tool

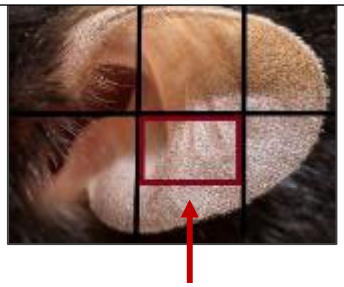
Figures 27b: Ear Notch/Punch with Long Tool

2. Ear Tag

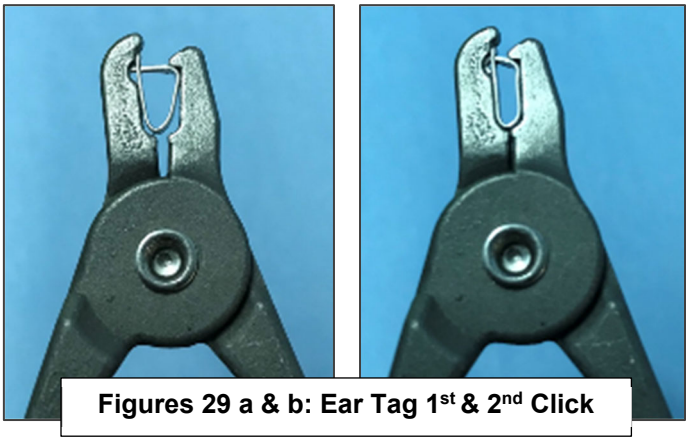
Ear tags can be purchased with numbers and/or letters. Correct placement of the tag makes them easy to read. Note, proper placement of the tag is important. If the tag is placed within the vasculature of the ear (too far inward) the mouse may experience itching and irritation, which can lead to infection. 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.

- Place the ear tag into the tagger making sure the hole lines up with the notch.
- Firmly scruff and restrain the mouse in your non-dominant hand. As with ear notch/punch, the head should be immobile to avoid tearing the ear tissue.
- Position the ear tagger on the ear.
- Aim for the middle third, avoiding the vasculature, and towards the lower ½, so the tag hangs appropriately after placement *(Figure 28)*.
- Firmly squeeze the ear tagging tool until the tag is closed, you should feel 2 clicks (*Figures 29 a & b)*.

Figure 28: Ear Tag Placement



Tag within this area



Figures 29 a & b: Ear Tag 1st & 2nd Click

Please note other forms of identification not discussed here:

- Long-term
    - Microchip
    - Tattoo
    - Toe clips (*see IACUC Standard*: *Standard on Rodent Identification*)
- 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 DCM Training & Care Assurance Team at DCMTraining@unc.edu. You may send other 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. Please see **the UNC Guidelines for Rodent Blood Withdrawal** for more details on the techniques described below: Standard for Rodent Blood Withdrawal and Tail Biopsy

- **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 (Figure 31) 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

- Scruff the mouse firmly and be sure that the head is restrained.
- Locate the facial landmark on the mouse (hairless area shown in *Figure 30*). The puncture site is slightly behind and above this area. It is usually more easily see in white mice.
- Align the lancet caudally to the mandible and firmly and swiftly insert the point perpendicular to the vein and collect the blood with a tube (*Figures 32 a & b*).
- 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 and/or circle in the cage. If this happens, it must be humanely euthanized.

Figure 30: Submandibular Bleed

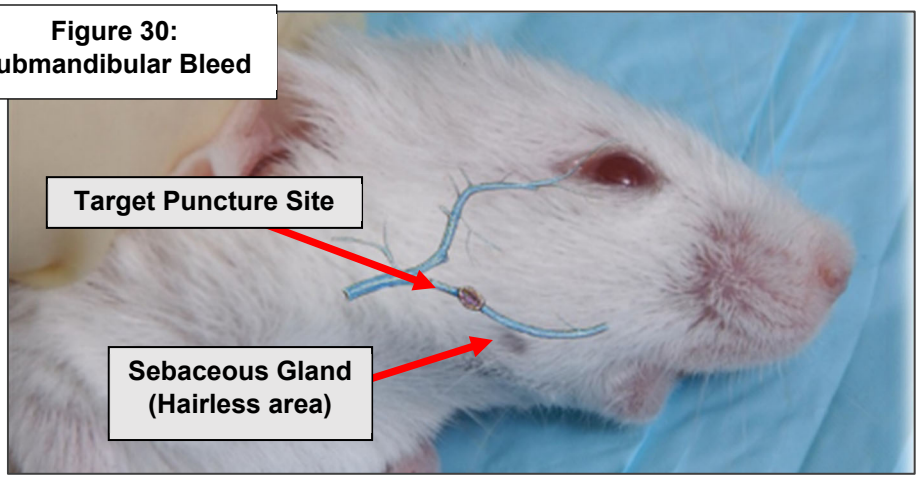
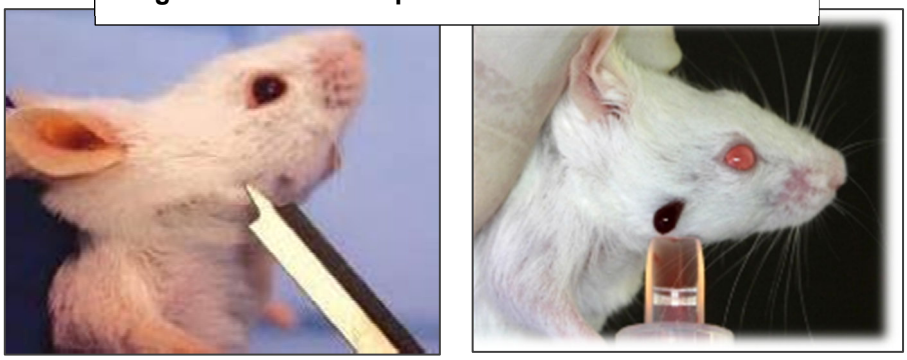


Figure 31: Lancet

Figure 32 a & b: Venipuncture and Blood Collection



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 33: Saphenous Vein

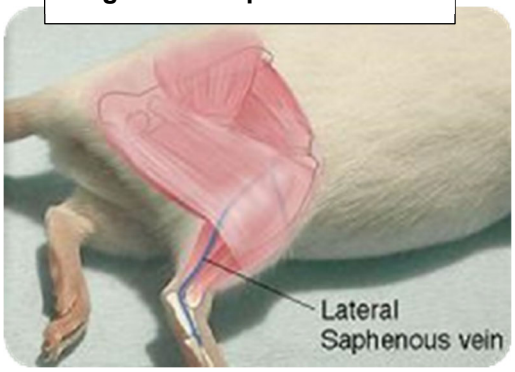


Figure 34 a & b: Saphenous Blood Collection

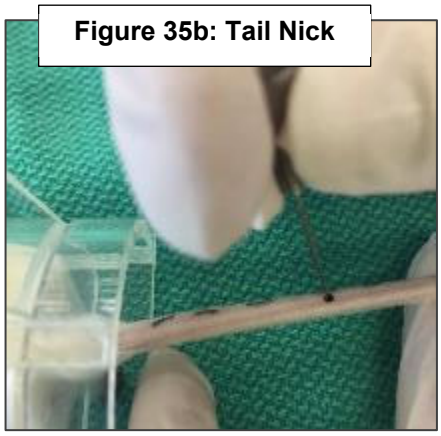
3. Tail Nick Bleed (Artery / Vein)

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 and mark the 12 o' clock position with a marker *(Figure 35a).*
- Locate the left or right lateral vein.
- Start midway up the tail and nick the artery or vein by inserting a lancet or the bevel of a needle perpendicular to the tail *(Figure 35b).*
- Gently twist and rotate the needle within this small hole to allow a few drops of blood to come out. You can milk the vein from the base towards the insertion site to retrieve a few additional drops if necessary.
- You may collect blood with micro capillary tubes, a micropipette or various microtainer collection tubes. Apply pressure to stop bleeding.

Figure 35a: Tail Nick

Figure 35b: Tail Nick



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: Standard for Rodent Blood Withdrawal and Tail Biopsy

1. Place animal in approved restrainer
2. Place the tail on a clean work surface (i.e. paper towel).
3. Using a fresh scalpel blade, cut 1-2 mm* of the tip of the tail.
4. Apply firm pressure straight down on the tail to cut the tissue in a single motion *(Figure 36)*. Avoid using the curved edge of the blade to prevent "sawing" the tail.
5. You can milk the vein from the base towards the tip of the tail to collect the necessary amount of blood.
6. Apply gentle pressure to the wound with a clean gauze pad or paper towel until the tail stops bleeding. A styptic powder can be applied to aid in stopping the bleeding.
7. 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.

**Never cut more than 4mm over the lifetime of the animal without IACUC approval. Bone can NEVER be exposed as a result of this procedure.*

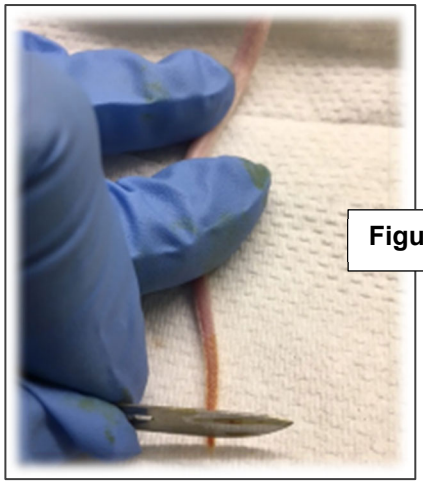


Figure 36: Tail Clip

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 the DCM Training & Care Assurance Team or DCM 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, gently direct the tube towards the back of the eye socket and rotate it to puncture the sinus and collect blood *(Figures 37 & 38).*
- Following blood collection, hold the eyelids closed to allow the punctured blood vessel to clot and apply ophthalmic ointment to the eye.

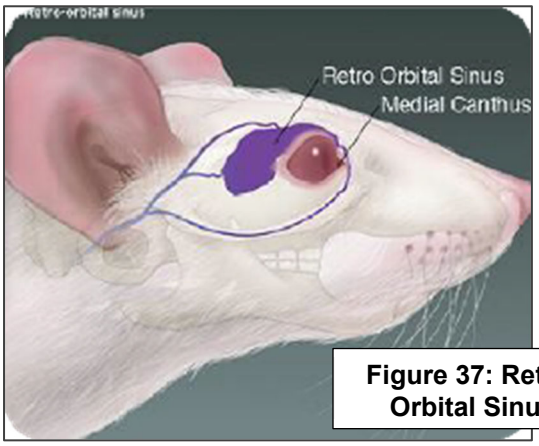


Figure 37: Retro-Orbital Sinus

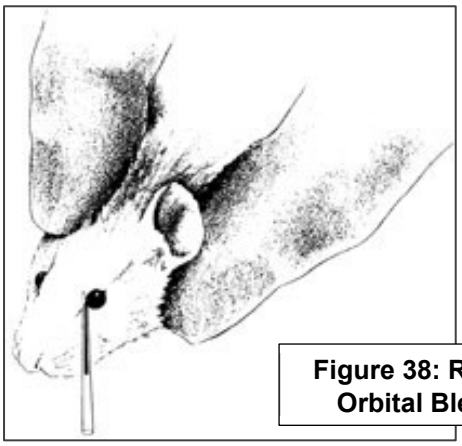


Figure 38: Retro-Orbital Bleed

6. Cardiac Puncture

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

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

1. Place the animal in dorsal recumbency on a flat, firm surface.
2. Hold the animal securely by placing the fingers immediately below the xiphoid process, or on the abdomen to prevent the mouse sliding.
3. Break the seal of the syringe and then insert the needle bevel up, going beneath the ribcage and puncturing the diaphragm.
4. 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 40)*. Small needle adjustments can be made under the skin, but care should be taken to avoid rapid movements to prevent organ laceration.
5. 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.
6. Ensure euthanasia by immediately performing a secondary physical method.

Figure 39: Cardiac Puncture Location

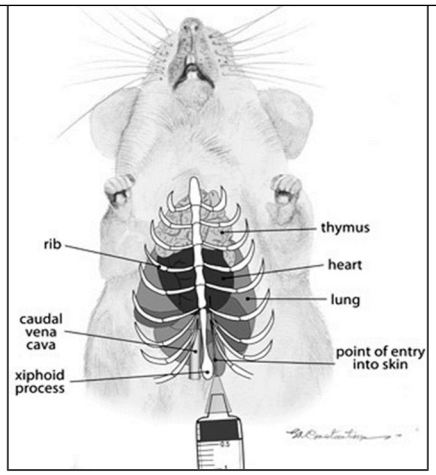


Figure 40: Cardiac Puncture

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 Standard on the IACUC website for more important considerations for euthanasia as well as information on neonate euthanasia. Standard on Rat and Mouse Euthanasia

Euthanasia Methods

There are 4 primary forms of euthanasia that are used on mice at UNC: 1) CO_2 euthanasia, 2) inhalational anesthesia, 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 3 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]
4. Collection of vital organs.

Note: In addition to DCM 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 (CO2)

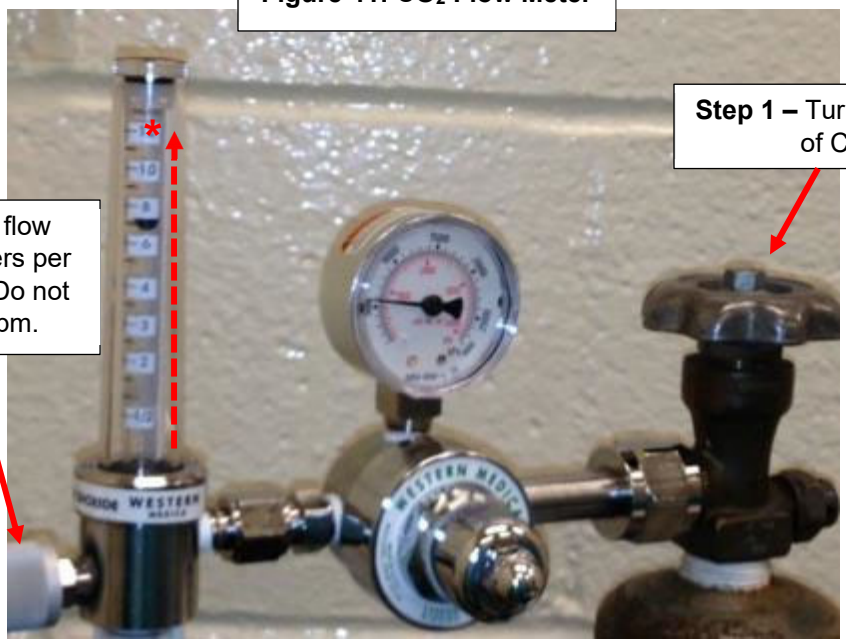
CO2 must be supplied using a compressed gas tank. The use of dry ice as a source of CO2 for euthanasia is not permitted. The 2020 AVMA Guidelines on Euthanasia recommends that the gradual displacement rate of CO2 into the euthanasia chamber should be 30-70% to minimize pain and distress. All calculations described below are for a DCM shoe box style rat cage at 45% displacement.

Note: DCM procedure rooms have dedicated CO2 euthanasia chambers equipped with acceptable flow meters. Investigators who wish to perform CO2 euthanasia outside of DCM facilities must adhere to all of the following principals and must purchase the same equipment utilized by DCM. 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/store/ (part number: 89012-426). To purchase appropriately sized euthanasia chambers, contact DCM at (919-843-7992).

1. Invert the cage before placing animals in, to remove residual CO2 that may be left from previous users.
2. Remove each animal from the housing chamber and place into the euthanasia chamber. Never place the housing chamber into the euthanasia chamber. Never pre-charge the 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. 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.
4. 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 DCM Shoebox style RAT cage: 12 liters per minute (lpm)
    b. Standard DCM Shoebox style MOUSE cage: 2.8 liters per minute (lpm)
    c. Other CO2 Chambers: Use the following formula to calculate the appropriate flow rate: Height x width x length / 61 = liters x 0.45 = flow rate/minute (units = cm)
5. 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.

6. 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. Refer to the full Standard for more details on acceptable physical methods.
7. 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.
8. Place dead animals into a non-PVC containing bag. DCM provides these bags in a variety of sizes. Label the bag with the IACUC protocol #. Seal the bag securely. Place the bag with dead animal(s) into the DCM carcass freezer available in each animal facility. Please see the Policy on Rodent Carcass Disposal for more information. Standard on Disposal of Rodent Carcasses
9. Disinfect the euthanasia chamber bottom after each use.

Figure 41: CO_2 Flow Meter



2. Inhalant Anesthetics (e.g. Isoflurane)

This occurs when anesthetic agent(s) are 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 placing two to three pieces of absorbent material on the bottom of the chamber (isoflurane drop method). Add approximately 3-5mls 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.
    a. Note – When using a vaporizer, anesthetic chamber cannot be pre-charged. See the "DCM Isoflurane Vaporizer Training" section on the DCM website for more details.

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.
    a. 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 42: Inhalational Vaporizer

Figure 43: 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 DCM 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 unanesthetized euthanasia, with requirements for each described below. Note, LACs are not allowed to certify others for this technique, training must be done by DCM veterinary services or the DCM Training & Care Assurance 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 ordered through DCM Veterinary Services. To order multiple bottles or purchase single bottles, contact Rosa Edsall at rbarrera@unc.edu

Braintree Scientific
Phone: 781-917-9526
Website: www.braintreesci.com
Description: Instruments, lab equipment, isothermal pads, tattoo paste

Fisher Scientific
Phone: 800-766-7000
Website: www.fishersci.com
Description: Lab equipment, chemicals, instruments, pharmaceuticals

Covetrus (formerly Henry Schein)
Phone: 800-872-4346
Website: https://northamerica.covetrus.com (redirects from www.henryscheinvet.com)
Description: Veterinary supplies, instruments, pharmaceuticals
Needs Vet License or Researcher DEA license

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

Med-Vet International
Phone: 800-544-752
Website: http://www.shopmedvet.com
Description: Veterinary supplies and instruments (discounted)
Need Vet License

National Band and Tag
Phone: 859-261-2035
Website: https://www.nationalband.com/
Description: ID tags, ear tags

Patterson Veterinary
Phone: 800-225-7911
Website: www.pattersonvet.com
Veterinary supplies, instruments, pharmaceuticals including Pentobarbital
Needs Vet License or Researcher DEA license

Plas Labs
Phone: 800-866-7527
Website: www.plas-labs.com
Description: Rodent restrainers, equipment

Roadrunner Compounding Pharmacy
Phone: 1-877-518-4589

Roboz
Phone: 800-424-2984
Website: www.roboz.com
Description: Specialize in instruments

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

Vetamac
Veterinary anesthesia service and manufacturer
Phone: 800-334-1583

Southern Anesthesia Surgical Inc.
Phone: 800-624-5926
https://www.sasrx.com/
This is a human source company that has a Veterinary division, will set up an account without a vet license.

DCM Vet Services
*If you need help finding and/or ordering drugs/veterinary supplies
Phone: 919-966-2906
Email: Rosa Barrera: rbarrera@email.unc.edu

UNC Physics Dept Instrument Sharpening
*Note: they do not sharpen to surgical grade requirements; will do guillotines Phone: 962-1183
Phillips Hall, Room 115, walk-ins are welcome https://physics.unc.edu/instrument-shop/

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McGill University
University of Texas Medical Branch at Galveston

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