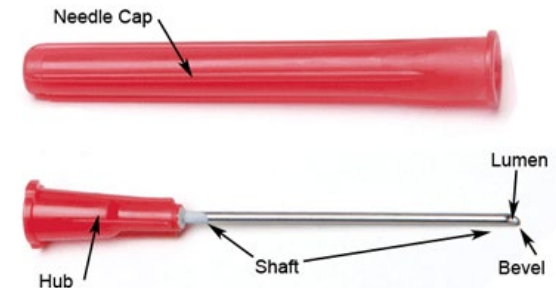
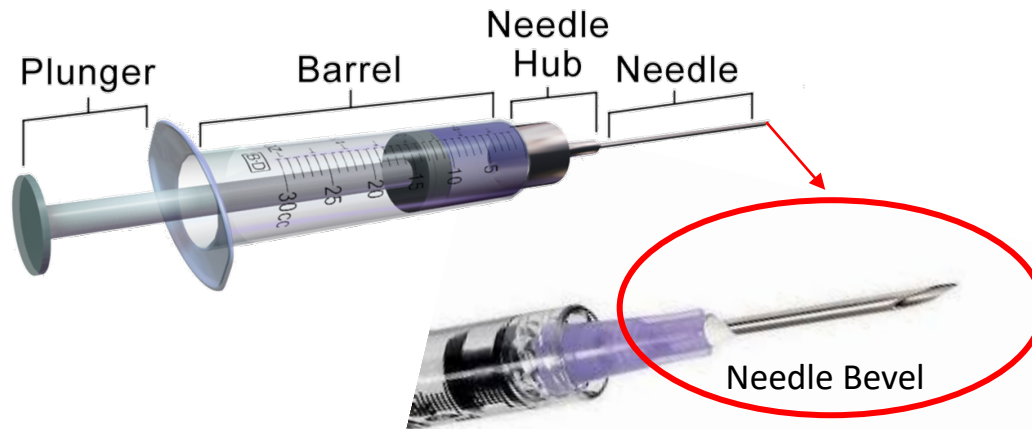


# Rat Handling & Techniques

*A Techniques Guide presented by the DCM Training & Care Assurance Team*



# Syringe Anatomy & Use



## Guidelines

- Do not touch the shaft or bevel of the needle before inserting into the animal (introduces bacteria)
  - Don't drag needle on bench/hood surface (use aseptic procedure)
- Change the needle or sterilize between animals
- Most procedures require you to insert the needle "bevel up" to prevent increased trauma
- Some procedures require aspiration prior to injecting (check for blood in hub)
  - MUST be able to aspirate and inject WITHOUT readjusting your hand on the plunger or excessively moving the tip of the needle
  - In the event you aspirate and see blood (and are not trying to inject in a vein/blood vessel), pull your needle out, reinsert, and try again.



# Restraint

## How to pick up a rat

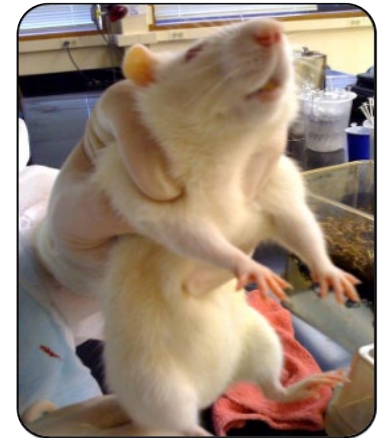
1. Grasp the animal at the base of the tail and lift, immediately supporting the rat's weight on your arm, hand, or place them on a stable surface. Arm support helps them relax. You may use Tyvek sleeves if you prefer more of a barrier between your arm and the rat but keep in mind that sleeves are made of smoother material that can make the rat feel insecure.
2. Never pick up a rat by the end/tip of the tail and do not dangle them by the tail. This hold is safe for very short periods of the time, and care must be taken as the tail is easily broken and/or stripped of skin.



## Two Restraint Options

### **Basic/Four-finger/Roller-coaster hold\***

While holding the tail base, place your non-dominant hand on the rat's back near the base of the tail and press down gently. Maintain pressure on the back and place the head between your index and middle finger, as close to the base of the fingers as possible. Then place your thumb and last 2 fingers along the body to support the chest. **DO NOT SQUEEZE** the thorax! Grasp the tail base with your other hand to support the body.



### **Shoulder/Two-finger hold\***

Grasp the tail base with the dominant hand and gently pull the rat backwards. With the other hand, place the thumb and index finger on the shoulder blades, pushing together until the forelegs are crossed (either leg can be on the top). Backward tension must be maintained on the tail, gently stretching to maintain upward lift of the shoulders.



\*Tail pressure must be applied with either hold – pin it around the wrist onto the benchtop (tail facing away from the body) or press the rat against the handler to keep the whole body restrained.

# Intraperitoneal Injection

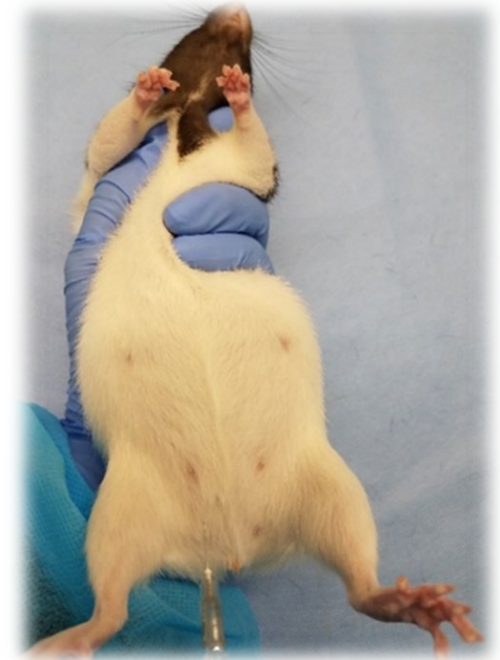
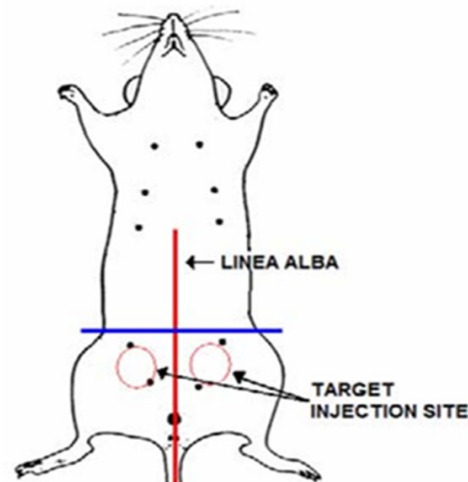
**Definition:** An injection into the lower abdominal cavity, penetrating the peritoneum of the animal.

## How?

1. Scruff and hold the rat in dorsal recumbency (on the back/belly facing upwards) 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.
2. Insert the needle bevel up in a position below the bend of the knee, to either side of the midline. This allows for a safe injection avoiding the bladder and other organs such as the liver or spleen.
3. Angle the needle approximately 45° to the body. Aspirate - if no blood is seen in the needle hub, inject slowly. If blood is seen, remove the needle and re-insert, aspirate, then inject if no blood is observed.

**Abbreviation?** IP

**Aspirate?** Yes



# Subcutaneous Injection

**Definition:** An injection administered beneath the skin & above the underlying muscle.

## How?

1. Place the rat on solid surface, cup the butt of the rat with your non-dominant hand and use your thumb and index finger to scruff the rat to tent the skin. Press the head towards the surface for maximum restraint.
2. Insert the needle bevel-up into the pocket or 'skin tent' you created by scruffing. This technique requires manipulating the syringe with one hand.
3. Aspirate - if no blood appears in the hub of the needle, inject slowly. If blood is seen, remove the needle and re-insert, re-aspirate, then inject if no blood is observed in the hub.
4. Large amounts of fluids may be administered but should be split over multiple locations to avoid distending the skin. The medium should be warmed to body temperature if giving more than a small amount so the rat does not become hypothermic.

**Aspirate?** Yes

**Abbreviation?** "SC" or "SQ" or "Sub Q"

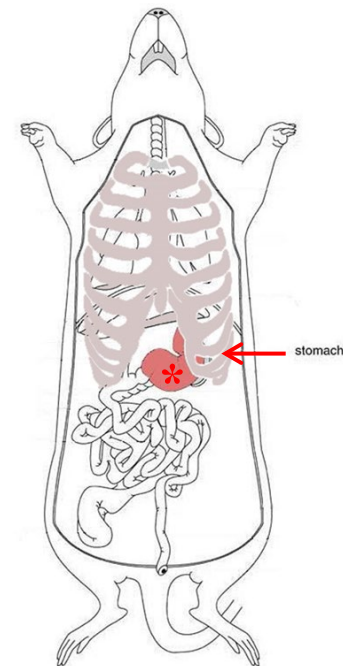
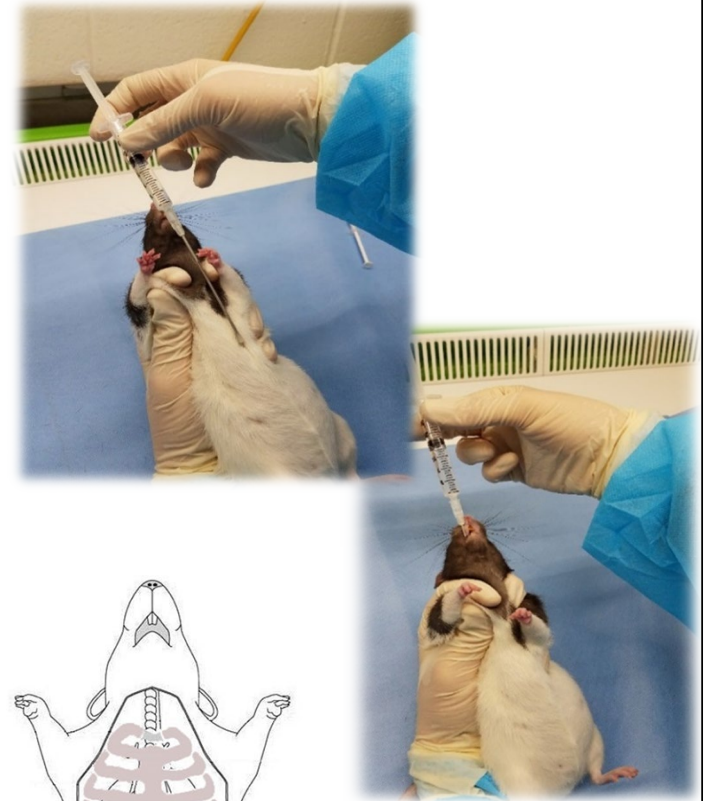


# Oral Gavage

**Definition:** A blunt, ball-tipped gavage needle is used to deliver substances directly to the stomach bypassing swallowing.

## How?

1. Firmly restrain the rat and measure the needle before inserting. The needle should measure the distance from the corner of the mouth to the xiphoid process (near last rib).
2. Insert the gavage needle into the mouth at one side 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 rat's head back, so that the neck is in a straight line.
3. 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 rat to swallow. If the rat struggles or the needle will still not pass any further, stop, withdraw the needle, and start over.
4. 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.



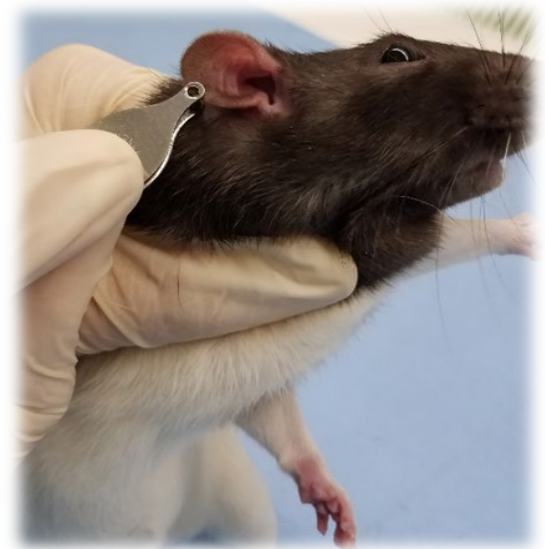


# Ear Notch/Punch

**Definition:** An identification and/or tissue harvesting method where a small hole is punched into the ear of the animal using a metal tool.

## How?

1. Firmly restrain the rat's head. The head should be immobile to avoid tearing the ear tissue.
2. Place the flat side of the ear notching tool on the ear so that you can see the area you will be punching.
  - a. Notch the outer edge of the ear to avoid the vasculature and excessive bleeding.
3. Firmly and quickly press down to punch the ear. Collect the tissue for genotyping if needed.

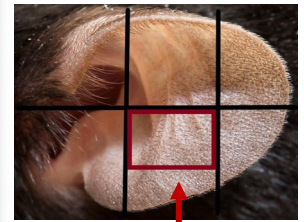


# Ear Tag

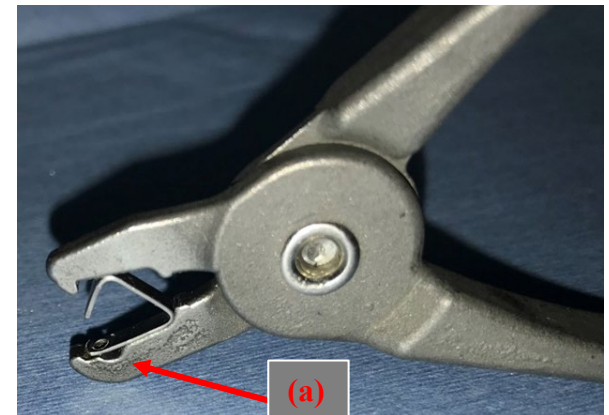
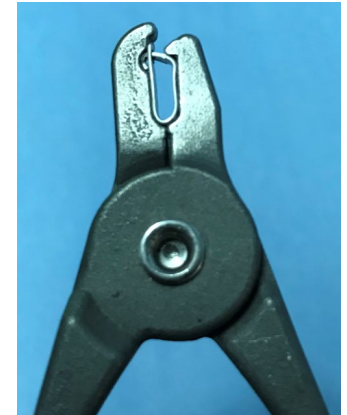
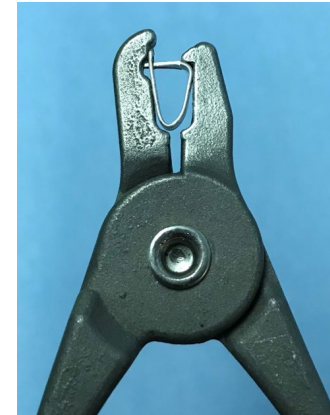
**Definition:** An identification method in which metal tags containing letters and/or numbers are placed in the ear.

## How?

1. Place the ear tag into the tagger making sure the hole lines up with the notch **(a)**.
2. Firmly restrain the rat's head with your non-dominant hand. As with ear notch/punch, the head should be immobile to avoid tearing the ear tissue.
3. Position the ear tagger on the ear.
4. Aim for the middle third, avoiding the vasculature, and towards the lower  $\frac{1}{2}$ , so the tag hangs appropriately after placement.
5. Firmly squeeze the ear tagging tool until the tag is closed, you should feel 2 clicks.



Tag within this area



0 cm 1  
|-----|



# Intramuscular Injection

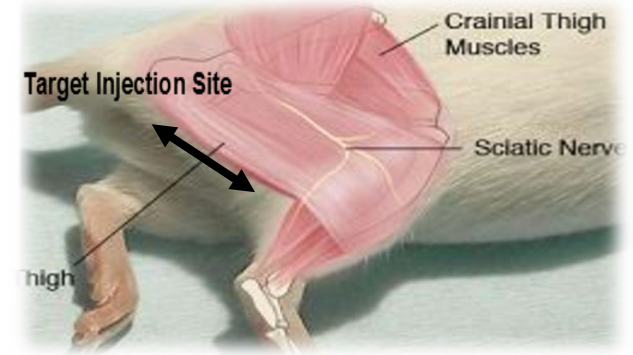
**Definition:** An injection into the muscle mass of the posterior thigh of the mouse. It is only used when other routes are not appropriate, as it is a potentially more painful injection.

## How?

1. The muscle mass running along the back of the leg is used. Care must be taken to avoid the sciatic nerve and the femoral vein, artery, and nerve.
2. Hold the rat against your body in the palm of your non-dominant hand. Press the rat into your body and roll the rat toward you with the belly facing away from you. Extend one of the rear legs outward with two fingers of the hand the rat is resting in.
3. 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. If blood is seen, remove and re-insert the needle and try again.

**Aspirate?** Yes

**Abbreviation?** "IM"



# Intravenous Injection

**Definition:** An injection into one of the lateral tail veins.

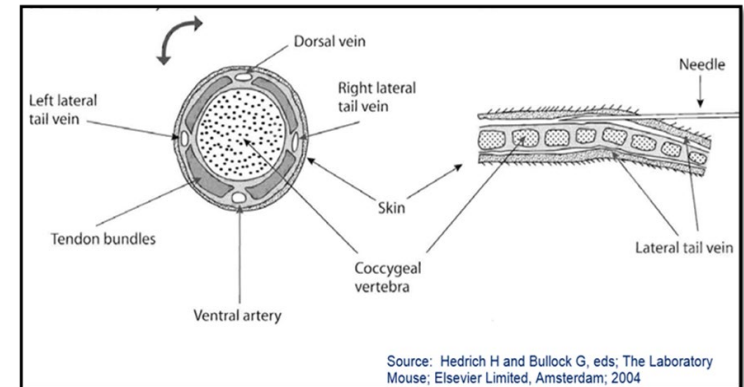
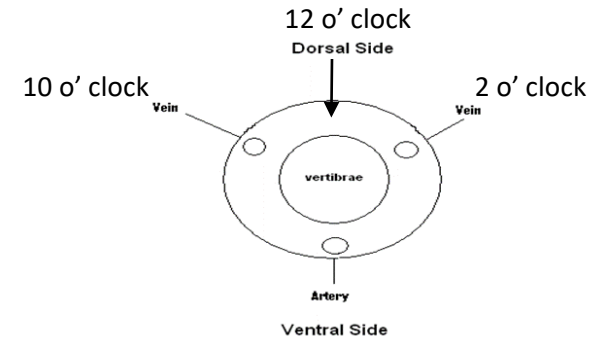
*\*One of the most difficult injection techniques to master\**

## How?

1. Place the rat into a restrainer and 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, then locate the left and right lateral tail veins, in the ~10 and 2 o' clock positions.
2. 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.
  - a. Needle should be almost parallel to the orientation of the tail and inserted shallowly to prevent passing through the vein.
3. 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.
4. 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.

**Aspirate?** No

**Abbreviation?** "IV"



# Tail Nick

**Definition:** A nick made into the tail vein or artery with a needle to obtain a small volume of blood (a couple of drops).

## How?

1. Place the rat in a restrainer and mark the 12 o' clock position with a marker.
2. Locate the left or right lateral vein.
3. 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.
4. 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.
5. You may collect blood with micro capillary tubes, a micropipette or various microtainer collection tubes. Apply pressure to stop bleeding.



# Tail Clip (for bleed or genotyping)

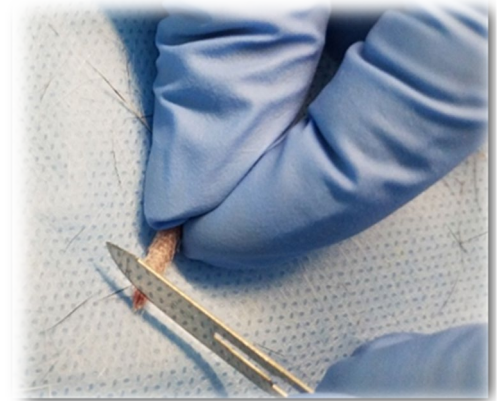
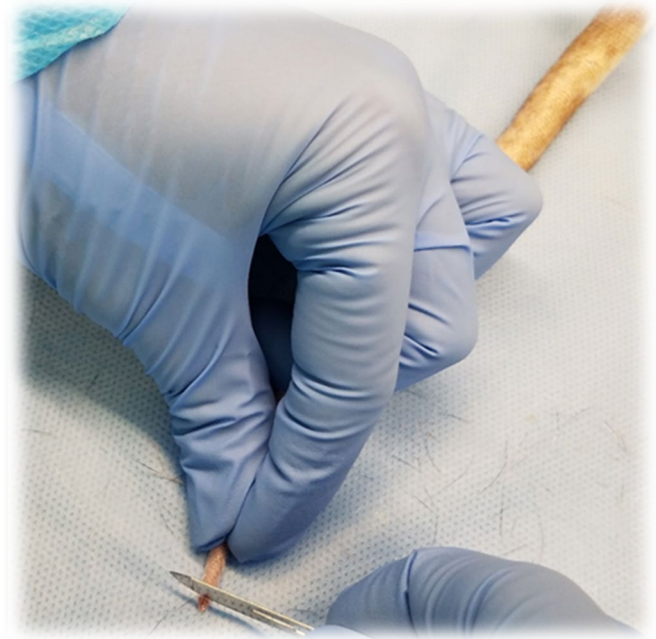
**Definition:** A method of collecting tissue for genotyping and/or blood by clipping a small fleshy portion of the tail.

## How?

1. Place animal in 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.
  - a. Apply firm pressure straight down on the tail to cut the tissue in a single motion. Avoid using the curved edge of the blade to prevent “sawing” the tail.
4. You can milk the vein from the base towards the tip of the tail to collect the necessary amount of blood.
5. Apply gentle pressure to the wound with a clean gauze pad or paper towel until the tail stops bleeding.
  - a. A styptic powder can be applied to aid in stopping the bleeding.
6. Return the animal to its cage only after bleeding has stopped.

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

*Please see the Standard for Rodent Blood withdrawal and Tail Biopsy on the IACUC website*

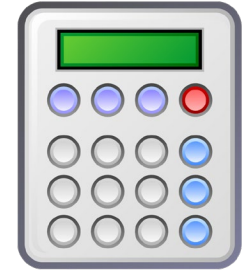




# Anesthesia Injectables

**Definition:** An injectable form of anesthesia which will cause the animal to be unconscious for a procedure or surgery.

1. Weigh the animal to calculate the appropriate drug dosage.
2. Administer IACUC protocol-approved anesthetic drug(s) based on weight and correct dose, via the approved injection route.
3. Place animal into a clean, empty cage with no bedding, no other animals, and on a heat source.
4. 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.
  - a. Care should be taken when using heat sources, to avoid overheating and burns.
5. 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.
6. 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. No response from all 4 feet is required prior to performing painful procedures.
  - a. Response = visible or tactile muscle movement, change in breathing pattern, etc.
7. 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).
8. Return animal to housing room only when fully awake and ambulatory.
9. Remember to document all anesthetic procedures as required.



**Required:** Heat source, eye lubricant, documentation, and negative toe-pinch reflex

**Toe pinch reflex present** = not ready for surgery!

**No toe pinch reflex** = ready for surgery!



# Cardiac Puncture

**Definition:** A terminal blood withdrawal directly from the heart performed under surgical plane of anesthesia or after death.

The following conditions must be met:

- Animal must be under a surgical plane of anesthesia when procedure is conducted – quickly perform a toe-pinch test on all 4 feet prior to executing this technique.
- Animal is NOT allowed to recover from anesthesia following the puncture.
- A secondary physical method is required to ensure death after blood collection is complete.

## How?

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 rat 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. 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.

