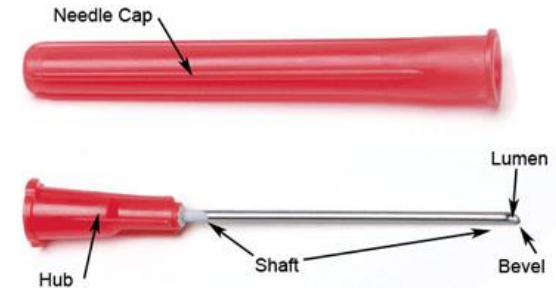
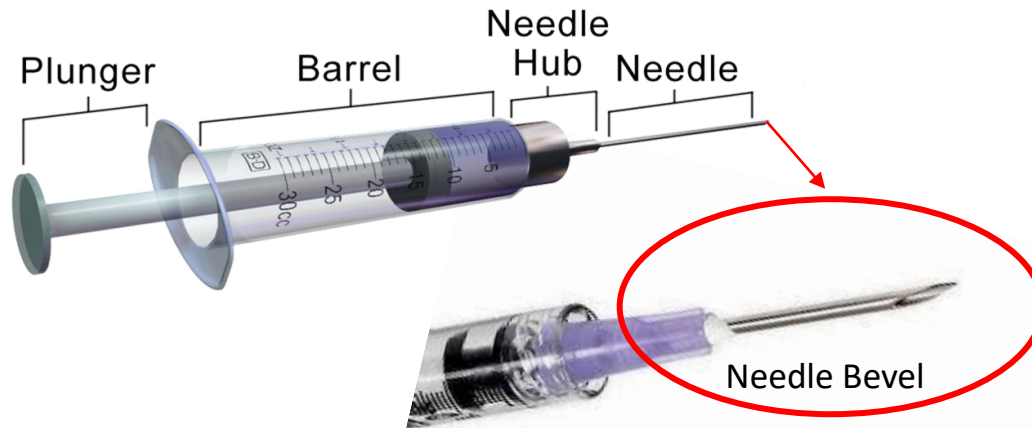


Mouse Handling & Techniques

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



Syringe Anatomy & Use



Guidelines

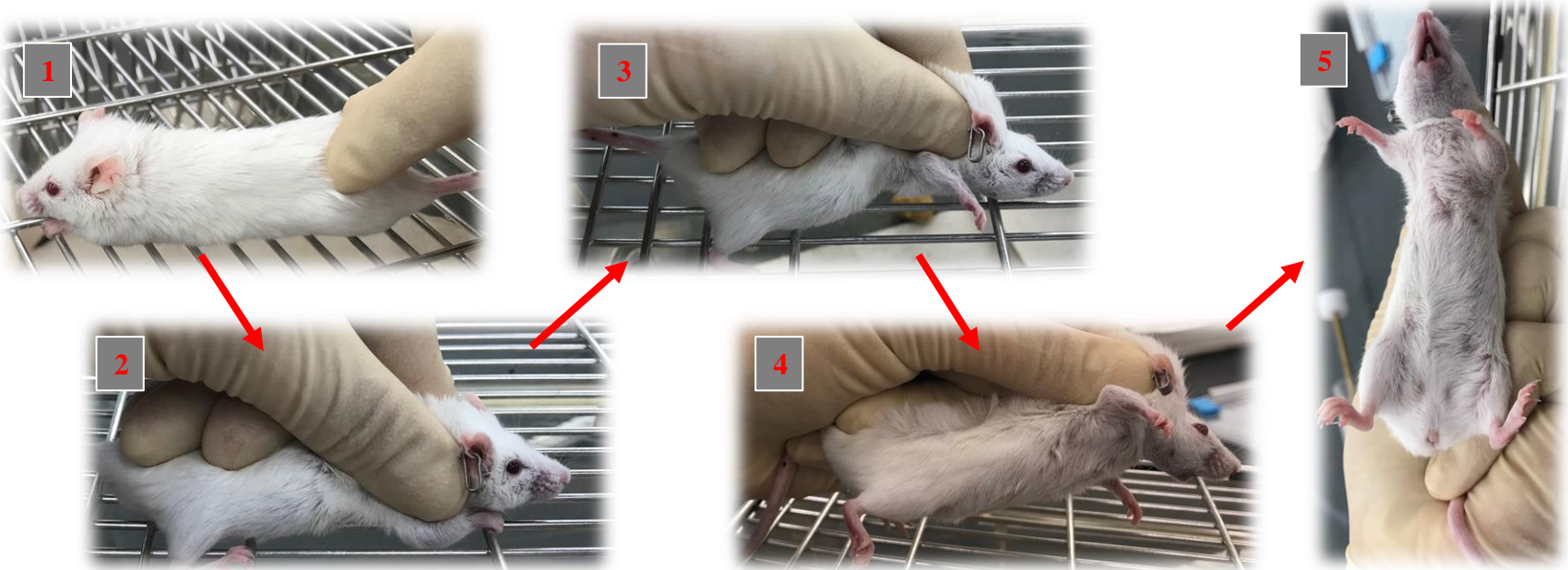
- Do not touch the shaft or bevel of the needle before inserting into the animal (introduces bacteria)
 - Don't drag needle on bench/hood surface (use aseptic procedure)
- Change the needle or sterilize between animals
- Most procedures require you to insert the needle "bevel up" to prevent increased 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 restrain:

1. Grasp the mouse gently but firmly at the base of their tail.
2. Immediately place the animal on a surface, do not let them dangle. Something graspable like a wire cage lid makes them feel more secure.
3. 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. Firmly pinch the loose skin straight up, lift the mouse, tug the tail towards your wrist and secure the tail with ring/pinky finger.



Intraperitoneal Injection

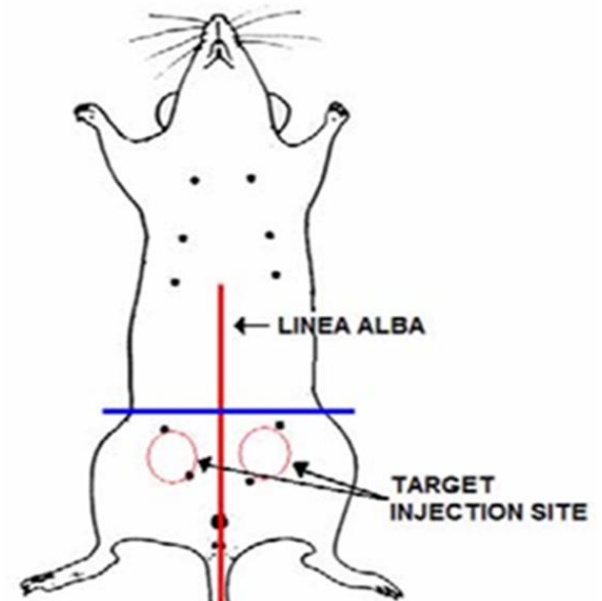
Definition: An injection into the lower abdominal cavity, penetrating the peritoneum of the animal.

How?

1. Scruff the mouse firmly and hold in dorsal recumbency.
2. Insert the needle bevel up in a position below the bend of the knees and to either side of the midline. This allows for a safe injection avoiding the bladder and other organs such as the liver or spleen. Also ensure the needle does not cross above the knees once inserted.
3. Angle the needle approximately 45° to the body. 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? IP



Subcutaneous Injection

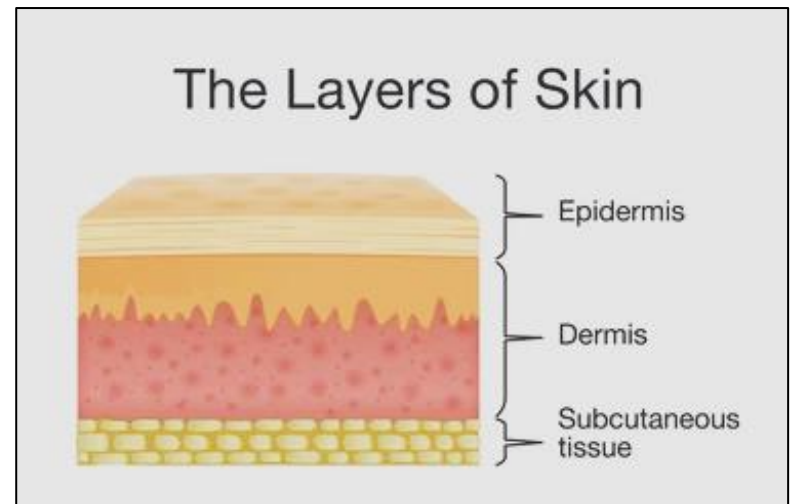
Definition: An injection administered beneath the skin & above the underlying muscle.

How?

1. 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.
2. Insert the needle into the skin tent and aspirate to check for blood. If no blood is seen in the syringe after aspiration, proceed with the injection. If you see blood, remove the needle and try above steps again until no blood is seen, then you can give your injection.

Aspirate? Yes

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

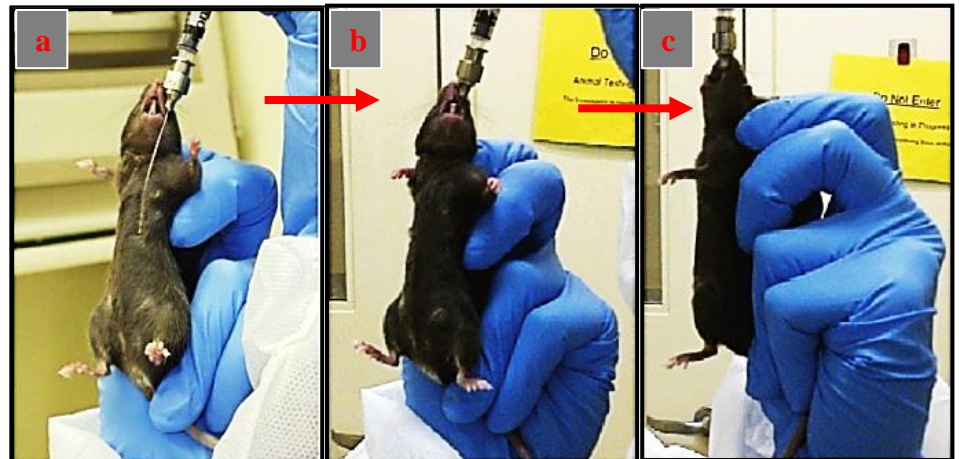
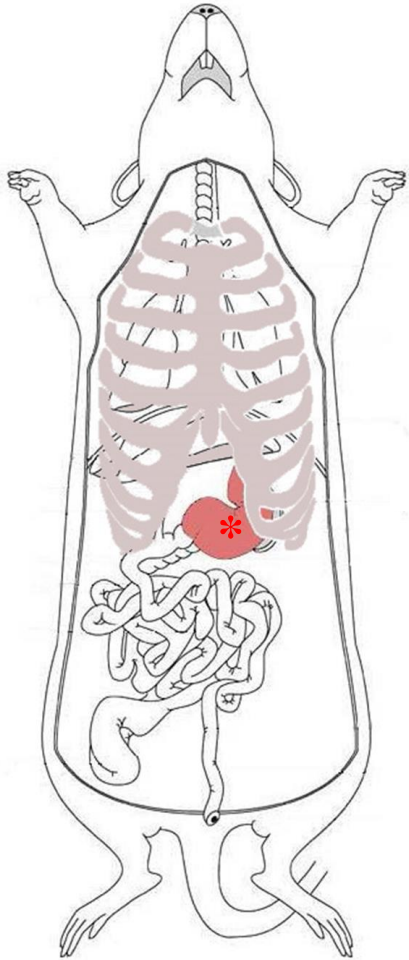


Oral Gavage

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

How?

1. Firmly restrain the 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).
2. 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.
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 mouse to swallow. If the mouse struggles or the needle will still not pass any further, stop, withdraw the needle, and start over.
4. 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.

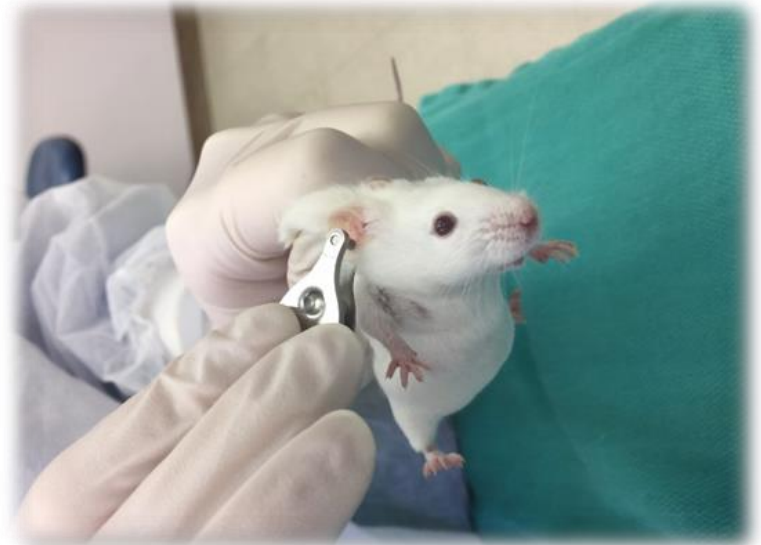


Ear Notch/Punch

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 scruff the mouse. 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.

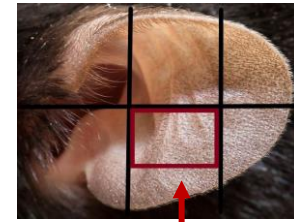


Ear Tag

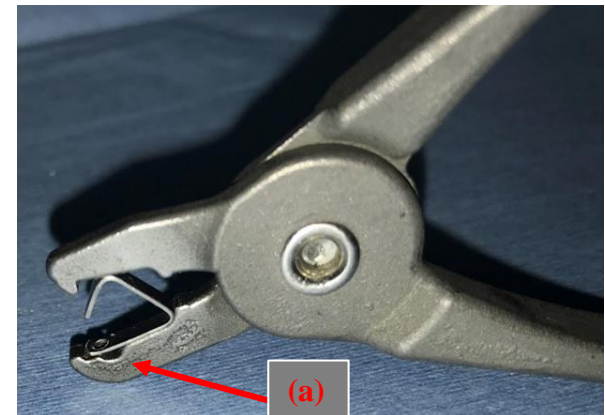
Definition: An identification method in which metal tags containing letters and/or numbers are placed in the ear.

How?

1. Place the ear tag into the tagger making sure the hole lines up with the notch **(a)**.
2. Firmly 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.
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



Intramuscular Injection

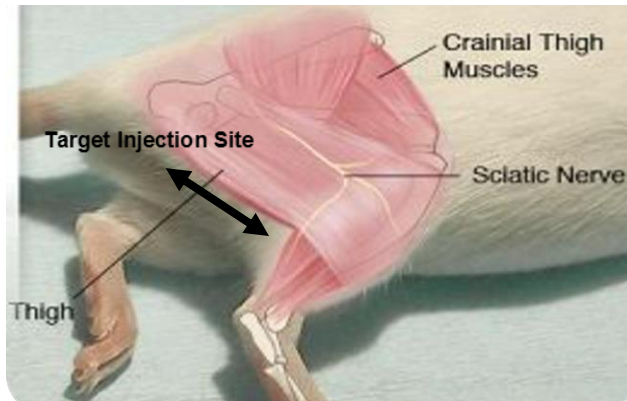
Definition: An injection into the muscle mass of the posterior thigh of the mouse. It is only used when other routes are not appropriate, as it is a potentially more painful injection.

How?

1. The muscle mass running along the back of the rear leg is used. Care must be taken to avoid the sciatic nerve, and femoral vein, artery, and nerve.
 - a. One person method w/o restrainer – Perform normal restraint and rotate mouse to expose rear leg.
 - b. One person method w/ restrainer – Gently pull the mouse using only the tail, while holding one of the rear legs through the top opening. Be very careful to not dislocate or twist the leg.
 - c. Two-person method – One person restrains the mouse, while the second person extends the rear leg and performs the injection.
2. 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”

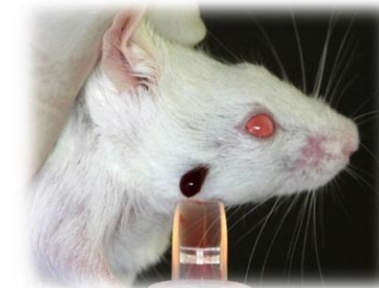
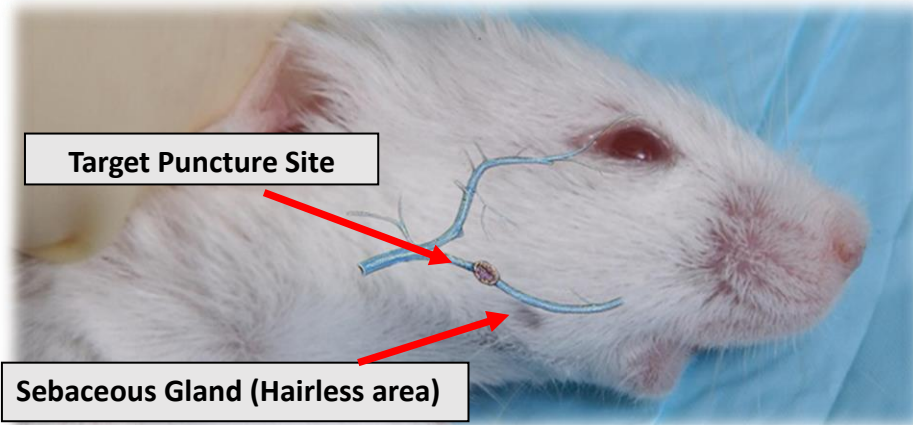


Submandibular Bleed

Definition: A way to obtain blood from a mouse by puncturing the area behind the hinges of the jawbones.

How?

1. Scruff the mouse firmly and be sure that the head is restrained.
2. Locate the facial landmark on the mouse (hairless area shown below). The puncture site is slightly behind and above this area.
 - a. More easily seen in white mice
3. 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.
4. 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.



Intravenous Injection

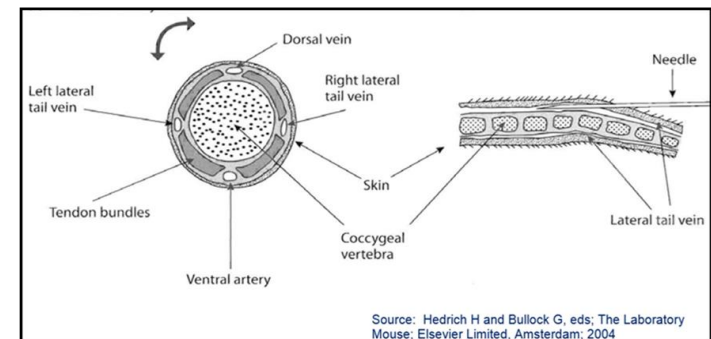
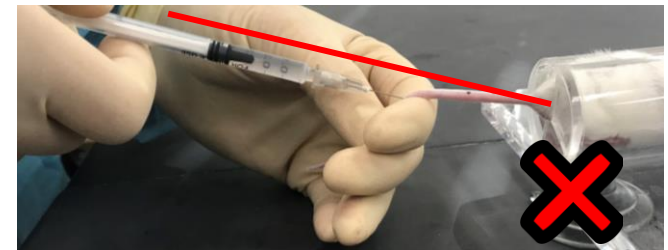
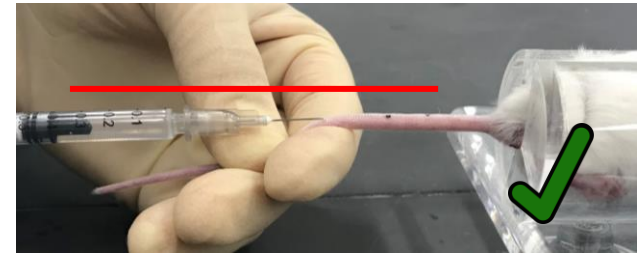
Definition: An injection into one of the lateral tail veins.

One of the most difficult injection techniques to master

How?

1. Place the 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.
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 mouse 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

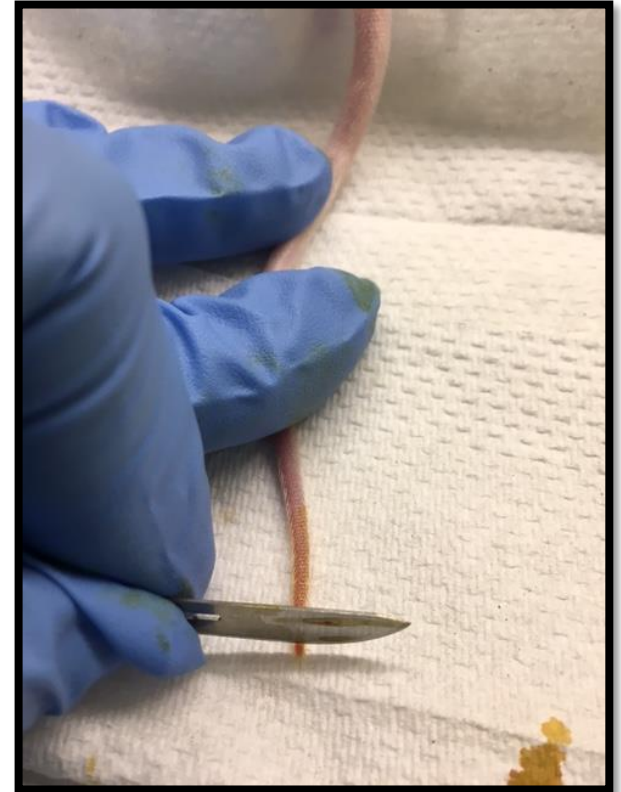
Definition: A method of collecting tissue for genotyping and/or blood by clipping a small fleshy portion of the tail.

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.***

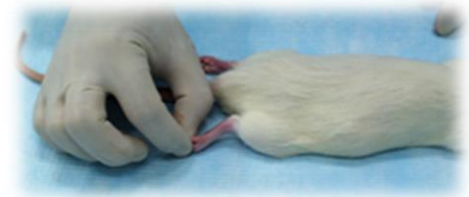
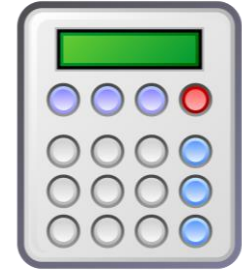
Please see the Standard for Rodent Blood withdrawal and Tail Biopsy on the IACUC website



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 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. 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.

