**Lateral Saphenous Vein**

Technique Suitable for Mice and Rats

**Method**

- Restrain the mouse or rat (see IACUC Guideline 8); this may require two people or a restraining device.
- Shave the hair covering the lateral saphenous vein with a scalpel blade (the vein is located caudal and lateral to the fibula and tibia).
- Clean the shaved area.
- Apply pressure around the leg above the stifle (knee) - this will help improve venous filling.
- Puncture the saphenous vein with a 25 gauge needle.
- Collect the blood accumulating over the incision using a hematocrit tube (see IACUC Guideline 1).
- Apply direct pressure to the incision for 1-3 minutes to facilitate hemostasis.
- Repeated blood samples may be obtained by removing the scab.

**Advantages**

- Serial blood collections may be obtained.
- This technique has the least potential to cause long-term pain and distress.

**Disadvantages**

- Blood collection is slow and may take 1 to 2 minutes to collect a sample.
- Requires specialized equipment and/or assistants.
- Requires personnel training for proficient use of this technique.
Potential Considerations

- Analgesia for rodents in pain.
- Sedation for rodents that are difficult to handle.

**Tail Vein**

*Technique Suitable for: Mice and Rats*

**Method: Mouse**

- Anesthetize the mouse (see [IACUC Guideline 15](#)).
- Clean the area over the tail vein 3 cm from the base of the tail; use an acceptable antiseptic scrub.
- Make a small transverse incision with a sterile scalpel blade, partially through the lateral tail vein.
- Use a hematocrit tube or blood-collection tube to collect the blood dripping from the incision (see [IACUC Guideline 1](#)).
- Apply direct pressure to the incision for 1-3 minutes to facilitate hemostasis.
- Subsequent blood samplings may be obtained in awake animals by removing the scab; if a new incision is required, the animal must be anesthetized.

**Method: Rat**

- Restrain the rat (see [IACUC Guideline 8](#)).
- Clean the area over the tail vein 3 cm from the base of the tail; use an acceptable antiseptic scrub.
- Apply heat (warm water, heat lamp, etc.) to the tail to facilitate vasodilation of the tail vein; take precautions to prevent thermal burns.
- Insert a syringe equipped with a 25 gauge needle at a 45 degree angle toward the vein.
- Apply negative pressure during needle insertion (this will help determine if the needle has entered into the vein).
- Use gentle negative pressure to collect blood - too much pressure will collapse the vein. (See [IACUC Guideline 1](#)).
- Apply direct pressure to the incision for 1-3 minutes to facilitate hemostasis.
Alternatively, a 25 gauge needle (without syringe) can be inserted into the tail vein; blood accumulating in the needle hub may be collected into a hematocrit tube.

Advantages:
- Serial blood collections may be obtained.
- If done correctly, this technique is not likely to cause long-term pain and distress.

Disadvantages:
- Anesthesia should be considered for this technique.
- Blood collection is slow in the mouse and may take 1 to 2 minutes.
- Temporary or permanent damage may occur to the vein during routine blood collection.
- Requires personnel training for proficient use of this technique.

Potential Considerations
- Analgesia for rodents in pain.
- Sedation or anesthesia for rodents that are difficult to handle.

Tail Sectioning
Technique Suitable for: Mice and Rats
Method:
- Restrain the mouse or rat (see IACUC Guideline 8); this will require a restraining device.
- Clean the tail with an appropriate antiseptic solution.
- With a sterile scalpel blade, make a transverse section through the long axis of the tail 2 mm from the tip.
- Use a hematocrit tube or blood-collecting tube to collect blood dripping from the sectioned tail (see IACUC Guideline 1).
- Massage the tail by passing the thumb and index finger from the base to the tip of the tail if blood flow is inadequate.
Apply direct pressure to the incision for 1-3 minutes to facilitate hemostasis; a suture may be required in some rats to close the surgical defect.

Repeated blood sampling may be obtained by two methods -- method selection is based on the length of time after the initial incision.

- 0 - 24 hrs.: Bleeding may be restarted by removing the clot; always try this method first...!!!
- > 24 hrs.: Follow the above protocol; however, make the new cut through the tail 1 mm from tip.

**Advantage:**

- Rapid, simple and easy.
- Multiple blood samples may be obtained from the initial tail excision by removing the scab.

**Disadvantages:**

- Requires equipment for rodent restraint.
- Multiple tail excisions may be required for repeated blood collections.
- The risk of causing pain and distress increases with repeated tail cuts; especially if the tail vertebrae are damaged.

**Potential Considerations:**

- Analgesia for rodents in pain
- Prevent pain and discomfort -- limit the number of tail incisions!!

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**Retroorbital Bleeding**

**Technique Suitable for: Mice and Rats**

**Method: Mouse**

- Anesthetize the mouse (see IACUC Guideline 15).
- Apply pressure to the external jugular vein caudal to the mandible with thumb, and gently elevate the upper eyelid with the index finger of the same hand.
- Insert a hematocrit tube into the medial canthus of the eye.
Gently direct the hematocrit tube in a ventrolateral direction until blood is obtained.

Once the desired amount of blood is obtained (see IACUC Guidelines Annex I), discontinue the external jugular pressure and remove the hematocrit tube.

Gentle pressure on the globe may be used to provide hemostasis.

Method: **Rat**

- Anesthetize the rat (see IACUC Guidelines Annex XV).
- Apply pressure to the external jugular vein caudal to the mandible with thumb, and gently elevate the upper eyelid with the index finger of the same hand.
- Break a small piece off the hematocrit tube.
- Insert the broken edge of the hematocrit tube into the conjunctiva of the mid-dorsal globe.
- Gently direct the hematocrit tube in a caudal and medial direction until blood is obtained.
- Once the desired amount of blood is obtained (see IACUC Guideline 1), discontinue the external jugular pressure and remove the hematocrit tube.
- Gentle pressure on the globe may be used to provide hemostasis.

**Advantage:** A fast way to collect blood.

**Disadvantages:**

- Requires the use of anesthesia.
- May cause ocular trauma.
- Cannot obtain multiple samples over a short period of time.
- Personnel training is required for proficient use of this technique.
- This technique may cause long-term pain and distress.

**Potential Considerations:**

- Analgesia for rodents in pain
- Limit the number of retroorbital bleedings per eye
References & Additional Sources Of Information


Blood collection in mice using the saphenous vein - An alternative to retroorbital collection

A pictorial description of the method can be viewed on the web (http://www.uib.no/vivariet/mou_blood/Blood_coll_mice_.html).