Guidelines for Rodent Blood Withdrawal and Tail Biopsy

Each laboratory must designate a Laboratory Animal Coordinator (LAC) who may train research personnel in their laboratory in various animal-handling techniques, including blood collection. The LAC must be certified by the Office of Animal Care and Use (OACU) or the Division of Laboratory Animal Medicine (DLAM) and demonstrate proficiency before training others within their lab. Alternatively, laboratory personnel may register for a hands-on techniques class here or one on one session with the OACU Training and Compliance team (966-5569).

- **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.

- **Acute or Single Blood Withdrawal**: The maximum survival amount of an acute blood withdrawal is 1% of the lean body weight. [eg; For a 20 gram adult mouse, no more than 4 X 50 ul micro capillary tubes (200 ul), may be withdrawn].

To facilitate blood collection, warm the rodent first. When using the tail veins or artery, you may dip the tail in warm water (45°C). The entire animal can be warmed with a carefully placed heat lamp for 5-10 minutes or by placing the housing cage on a circulating water pad. Alternatively, alcohol may be used initially as a vasodilator, but it should not be used on broken skin.

**MICE:**

1) **Submandibular:**
A relatively simple way to obtain blood from a mouse is to puncture the area behind the hinges of the jawbones. The superficial temporal vein is a large vessel positioned behind the eye, which can be traced backward to the temporal vein, the maxillary vein, and finally, the jugular vein.

Scrub the mouse and pierce the skin in the relevant area. A mouse bleeding lancet is strongly recommended for use. However, an 18 gauge needle may also be used. Information on the lancets and a video of this procedure may be seen by going to the following URL: http://www.medipoint.com/html/animal_lancets.html

The submandibular bleed is one of the easiest methods of collecting blood from a mouse. Your LAC may train in this technique or you can contact the OACU at 966-5569 to arrange for training.

2) **Saphenous Vein:**
This method of obtaining blood is often used when a series of small samples is required. Place the mouse in a conical tube and shave the caudal surface of the thigh. The saphenous vein can be seen in this area. It is advantageous to apply a lubricant to prevent wicking. Place a tourniquet above the knee and enter the vein with a 25 gauge needle. Microhematocrit and microvette tubes work well to collect the blood. This method of blood withdrawal does not require anesthesia, however, the method of
restraint is cumbersome. For detailed instructions and pictures of this procedure please visit http://www.uib.no/dyreavd/Vivarium-blood-sampling.pdf

3) Tail Artery / Vein (NICK):
Tail veins and artery can be used for serial bleedings. Use the central tail artery or lateral tail veins. Anesthesia is not required for tail nick. Start midway up the tail and nick the artery or vein (usually with a needle or lancet). You may collect blood with micro capillary tubes, a micropipette or various microtainer collection tubes. Move cranially 0.5 cm at a time applying pressure after the bleed.

RATS:

1) Tail Artery and Veins:
Blood may be withdrawn from the ventral tail artery using a plungerless syringe and a 22-gauge (or smaller) needle. When bleeding from either of the lateral tail veins, a 22-gauge needle is inserted into the vein. Let the blood drip into the collection vessel. A tourniquet placed at the base of the tail will facilitate bleeding. Anesthesia is not required but is highly recommended for ease of collection.

MICE AND RATS:

1) Tail Clip Bleed and/or Tail Biopsy for Genotyping:
Performed on (un)anesthetized or anesthetized animals depending on amount of tissue needed (see below):

   - Anesthesia is optional for the removal of up to 4mm from the tail tip. It is strongly recommended that no more than 2mm be removed at a time. Anesthesia may be used as a means of animal restraint and its use must be described in the approved animal care application.

   - Harvesting greater than 4mm requires written permission from the IACUC. Depending on the age of the animal, removal of greater than 4mm from the tail tip may involve cutting into the vertebral column. Therefore, anesthesia is always required when removing this much tail, irrespective of the age of the animal. The use of anesthesia must be described in the approved animal care application. Requests to perform tail biopsies or successive tail cuts totaling greater than 4mm without anesthesia must be scientifically justified and must receive IACUC approval prior to implementation.

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. See policy below.

1. Place animal in approved animal restrainer. (Experienced handlers may be able to perform technique in habituated rats with light or no restraint).
2. Remove any bedding material or feces from the tail. The tail tip must be disinfected with an approved disinfectant (i.e. Betadine)
3. Place the animal on a clean work surface.
4. Using a fresh scalpel blade, cut 1-2 mm of the distal tail at an angle perpendicular to the work surface.
5. Apply gentle pressure proximal to the collection site to occlude venous return and ease collection. Collect the blood in a suitable collection device.

6. Apply gentle digital pressure to the wound for 30-45 seconds with a clean gauze pad to stop any hemorrhaging. For persistent bleeding, apply a silver nitrate stick, styptic powder or a cautery pen to the wound to stop bleeding.

7. Return the animal to its cage only after bleeding has stopped.

8. Serial blood samples can be obtained over a short time frame by gently removing the scab without performing an additional cut.

9. 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.

10. This procedure should be performed only by individuals trained and certified in the technique and comfortable with rodent handling.

2) 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 OACU Training and Compliance team or DLAM veterinary services.

Alternating eyes for each bleeding is mandatory, and a week must separate each bleeding. A maximum of two (2) bleedings per eye is permitted. Maximum volume withdrawn within a two week period is 1.5% body weight. Orbital sinus bleeding requires training and must be performed on anesthetized animals only with IACUC approval.

"Following blood collection, the eyelids should be held closed for a few seconds to allow the punctured blood vessel to clot. It is also common practice to place a small amount of ophthalmic ointment into the eye following this procedure." excerpt from Laboratory Animal Technician Training Manual

3) Cardiac Puncture: Always a terminal procedure conducted under anesthesia! Cardiac puncture as a method of blood withdrawal permitted in all species provided the following conditions are met:
1. Animal is under a surgical plane of anesthesia when procedure is conducted.
2. Animal is NOT allowed to recover from anesthesia following the puncture.
3. If the animal is euthanized prior cardiac puncture, training and certification in the technique is not required.

A needle is inserted into the heart and blood is extracted until a sufficient volume is collected or the animal is exsanguinated. This procedure must be followed by a physical euthanasia method.