

ACCEPTABLE METHODS OF RODENT BLOOD WITHDRAWAL

Each laboratory must designate a Laboratory Animal Coordinator (LAC) who will be responsible for training 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 in blood withdrawal techniques. The prospective Laboratory Coordinator must contact the OACU office at 966-5569 to register for training classes.

- * **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 [eg; For a 20 gram adult mouse, no more than 4 X 50 ul micro capillary tubes (200 ul), may be withdrawn].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.

MICE:

1) Submandibular Bleeding:

A relatively simple way to obtain blood from a mouse is to puncture the area behind the hinges of the jawbones. Veins that drain blood from various parts of the face meet in this area and form the jugular vein.

Scruff the mouse and poke a small hole in the relevant area. A mouse bleeding lancet is strongly recommended for use. However, a 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>

The submandibular bleed is one of the easiest methods of collecting blood from a mouse. Please 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 poke 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/vivariet/mou_blood/Blood_coll_mice_.html

3) Tail Artery and Veins (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. 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:

i. 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.

MICE AND RATS:

a. Tail Artery and Veins (CUT): Performed on (un)anesthetized animals.

The IACUC has approved the tail cut method for both rats and mice to obtain blood. This method must be described in the animal use application and approved by the IACUC prior to use. See policy below.

Volumes less than **1%** of lean body weight:

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, wash with a surgical skin disinfectant (eg: Betadine) and rinse with water.
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.
8. Serial samples can be obtained over short time frames by gently removing the scab.
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. To be performed only by individuals trained in the technique and comfortable with rodent handling.

b. Orbital Sinus Bleeding:

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. DLAM veterinary technical staff or one of the OACU Training/Compliance Coordinators will be happy to demonstrate submandibular and tail artery/vein bleeding.

Alternating eyes with each bleeding is mandatory, and each bleeding must be separated by a week. A maximum use is two (2) times per eye is permitted. Maximum withdrawal within a two week period is 1.5% body weight. Orbital sinus bleeding requires training and must be performed on anesthetized animals only.

“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

c. Cardiac Puncture: A terminal procedure!

Cardiac puncture as a method of blood withdrawal permitted in all species, provided the following two 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