

**ANIMAL RESEARCH INVOLVING BLOOD COLLECTION**  
**POLICY & PROCEDURES**

The volume of blood collected in most mammals is generally not a problem. However, repeated blood sampling in mice, rats, hamsters, guinea pigs, small cats, birds and some fish can be problematic.

Although blood volume is rapidly restored in an animal after blood collection, a two-week “rest period” is needed for blood constituents (red blood cells, platelets, clotting factors, etc.) to be regenerated by the body. As a general rule, 1% of an animal’s body weight (measured in grams) can be collected in blood (measured in milliliters) within a 24-hour period, every 14 days. For example, 0.3 ml can be collected once every two weeks from a 30-gram mouse. Alternatively, 0.05 ml of blood can be collected hourly for 6 consecutive hours from a 30-gram mouse, every two weeks ( $0.05 \times 6 = 0.3$  ml).

If blood needs to be collected once a week, it is recommended that not more than 0.5% of the animal’s body weight be removed within a 24-hour period. For example, 0.15 ml can be collected once a week from a 30-gram mouse. This volume can be further divided if blood needs to be collected more frequently. For example, 0.03 ml can be collected once a day for five days from a 30-gram mouse, provided the mouse is given a one week (or greater) “rest period” before blood is collected again. The key to determining how much and how frequently blood can be drawn depends on the “rest period” between blood collections.

Below is a summary of sampling types and recommendations for rodents:

**Cardiac puncture (Rodent):** It is recommended that this technique be limited to terminal collections due to the difficulty of the technique and the danger of lung injury or cardiac tamponade and death. Safer alternative techniques are available for large volume collection. Cardiac puncture should be done only under general anesthesia.

**Retro-orbital sinus collection (Rodent):** This technique is a reliable method for serial blood sampling that will give reasonable sample volumes (particularly for rodents). Increasingly, however, there has been concern about discomfort likely to be caused by the procedure. It is not uncommon to observe some animals with hemorrhage, optic nerve damage and swelling in the eye after retro-orbital bleeding, even when performed by experienced people. Satisfactory alternatives to blood collection via retro-orbital are available and recommended whenever possible. Safer alternative techniques are available for large volume collection and should be done only under general anesthesia.

Only persons who have been properly trained and with established proficiency should perform retro-orbital bleeding. Before doing retro-orbital bleeding in animals that are to recover after anesthesia, a person’s proficiency should be established. Training will include instruction in the procedure, observation of technique by the OACM staff, and procedure follow-up.

This technique should only be performed under general.

No more than two retro-orbital bleedings should be performed per week on a single animal. Left and right eyes should be alternated when performing repeated bleedings. No more than six retro-orbital bleedings (three in each eye) should be performed in a lifetime.

The animal should be euthanized immediately if the eye is injured as a result of retro-orbital bleeding.

**Submandibular Nicking (Mouse):** Submandibular bleeding is safely performed in an awake animal using a bleeding lancet or a 21 – 25g needle. The mouse is restrained by gripping the skin over the back of the neck and holding the animal upright. The submandibular vein can be punctured at the posterior border of the mandible, just slightly towards the ear. Insert a lancet or the tip of a hypodermic needle into the bundle of muscles at the back of the cheek pouch. Once sufficient volume has been collected, apply pressure to the site for at least 20 seconds to stop blood flow. A full description of this technique, with photos, is available at <http://www.labanimal.com/labanimal/journal/v34/n9/abs/lab1005-39.html>

**Tail Clipping (Mouse):** If tissue analysis requires tail tip amputation in an adult animal, it should be done under general anesthesia. Care should be taken to take the smallest piece of tissue possible and stop bleeding by application of gauze pad or silver nitrate, if necessary. Tail clipping is acceptable in a younger animal (21 days or less than weaning age); it is only acceptable in an older animal when general anesthesia and justification is provided.

**Saphenous vein (Rodent):** The mouse is most easily restrained using a 50 ml conical centrifuge tube or a restraint device. Place the mouse in the tube head-first and extend the hind limb over the top edge of the tube, applying gentle pressure over the top of the knee joint. The saphenous is visible just above and below the knee joint. The sampling site may be prepared by gently shaving the hair from the site using a scalpel blade held perpendicular to the surface of the leg. The vein is then punctured using a lancet or a 25 g sterile hypodermic needle. After adequate volume is obtained, the operator releases the downward pressure on the leg against the restraint device; this is usually adequate to stop the bleeding. In the event that bleeding continues, place pressure again directly on the venipuncture site for 15 to 20 seconds.

**Tail Vein (Rodent):** This technique is generally performed in an awake mouse or rat. The animal is positioned in a restraint apparatus. Blood collection is easier if the tail is warmed with a heat lamp, a warm paper-towel compress, or a 50-C water bath. In a light-colored mouse, the lateral tail vein can easily be visualized; it is more difficult to see in a dark brown or black mouse. Use a 25 g needle with the bevel facing upwards, line up the needle with the vein and gently insert. This can be used for either blood sampling or for injections.

Exceptions to this policy must be scientifically justified and approved by the UVM IACUC.