Guidelines for Collection of Blood from Laboratory Animals

These guidelines have been developed to introduce investigative staff to procedures recommended for blood collection in laboratory animals. This document is intended to supplement hands-on instruction by an experienced member of your laboratory.

Blood Sampling

Collection of blood from laboratory animals is frequently necessary for a variety of experimental uses including determination of pharmacokinetics, antibody production, clinical pathology evaluation, etc. Blood may be collected from animals which are to survive the procedure or at sacrifice as a terminal event. Whereas there is no limitation on the amount of blood that may be collected terminally, the volume collected from animals surviving the collection is limited to prevent anemia and hypovolemia. As a general guide no more than 10% of circulating blood volume may be collected during any 14 day period from animals surviving the blood collection.

Adverse Effects

If too much blood is drawn too quickly or too frequently without replacement, animals may develop hypovolemic shock. In the longer term the removal of too much blood causes anemia, muscle weakness, increased susceptibility to cold and reduced exercise tolerance.

If 15% to 20% of the blood volume is removed, cardiac output and blood pressure will be reduced. Removal of 30% to 40% can induce shock and death.

It is extremely important to apply pressure to the blood collection site, especially when penetrating an artery, for at least 3 to 5 minutes post blood collection to prevent hematoma formation.

The Animal Care Services (ACS) limits survival blood collection to 10% of the animal’s circulating blood volume. The frequency of blood collection is dependent upon the volume collected. If the maximum volume, as specified above, is collected within a 24 hour period, blood may be collected once every two weeks. The monitoring of hematocrit, serum protein levels, and potentially replacement fluid therapy are indicated when more frequent and/or higher volume collections are scientifically necessary; however, scientific justification is required.

Mice

There are a variety of methods that are utilized to collect blood from mice. The techniques described below are recommended by ACS staff for survival:

- tail veins
- saphenous vein
- facial veins
- orbital venous sinus* requires anesthesia see alternative listed below
- terminal (cardiac) blood collection under anesthesia.
Although described below, orbital venous sinus blood collection is generally considered unnecessary and inappropriate.

As an alternative to tail amputation for blood collection in mice, ACS recommends using the saphenous vein (this website is Norwegian but includes generous pictures and an English description) or instead of retro-orbital puncture use the facial vein (This video clip was produced by a commercial firm and does include “advertising”. Its use here does not construe product endorsement). These techniques may be performed in conscious animals.

Lateral tail vein venipuncture

The veins located on the lateral aspect of the mouse’s tail are useful for collecting small volumes (< 0.1 mL) of blood. The technique for venipuncture is as described for IV injection except that a small volume of blood is aspirated into the syringe instead of injecting material. The use of a needle without a syringe, allowing the hub to fill with blood, and subsequent collection into a microhematocrit tube is useful when very small quantities of blood are needed. For small blood volume collection, the tail vein can be simply punctured with a small gauge needle. Free flowing drops of blood can be collected, as with the facial or saphenous vein techniques.

Orbital venous sinus collection

The sinus surrounding the globe of the mouse’s eye is a useful site for collecting larger volumes of blood, but the facial vein provides similar volume and does not require anesthesia. General anesthesia must be provided when collecting from this site. Under general anesthesia the mouse is grasped so that its back rests on the palm of your left hand (right hand if you are left-handed) with its head toward your thumb. The thumb is placed just lateral to the animal’s trachea so that the jugular vein on the same side as the eye from which you are collecting blood is occluded and the fur on the animals head is drawn into the palm of your hand. This causes the animal’s eye to propstose (bulge) slightly. Be careful not to occlude the trachea! A 50 μL sterile microhematocrit tube is directed into the medial canthus (junction of eyelids closest to the animal’s nose) of the eye rotating slightly as the tube is directed to a point directly behind the globe. Sufficient pressure must be applied to cut through the fibrous layer that surrounds the sinus. Blood flows through the tube and occasionally around the tube once the sinus has been penetrated. After blood collection, the tube is removed and the eyelids closed and a dry cotton pad is applied over the eye with gentle pressure to prevent retroorbital hemorrhage. In general blood should not be collected from the same eye more than 2 times, allowing at least 2 weeks between collections. An antibiotic ophthalmic ointment must be applied following bleeding.

The orbital venous sinus route has been commonly used by researchers in the past but has been observed to cause many adverse effects. Concern has therefore arisen because of these effects and their potential severity. Recently, however, other methods have been developed that meet the scientific requirements and also improve the welfare of the animals. Bleeding from the plexus/sinus must always be carried out under general anesthesia. There are serious potential adverse effects: (i) retroorbital hemorrhage resulting in hematoma and excessive pressure on the eye, which is almost certainly painful for the animal; (ii) any pressure required to stem persistent bleeding (e.g. by pressing on the eye) or pressure from a hematoma can lead to corneal ulceration, keratitis, pannus formation, rupture of the globe and micro-ophthalmia; (iii) damage to the optic nerve and other intra-orbital structures, which can lead to deficits in vision and even blindness; (iv) fracture of the fragile bones of the orbit and neural damage by the micropipette; and penetration of the eye globe itself with a loss of vitreous humour. Many
of these unwanted sequelae may stay undetected, being located deep within the orbit. Ref J. Appl. Toxicol. 21, 15–23 (2001)

**Cardiac puncture (diaphragmatic approach)**

Cardiac puncture is the preferred technique for terminal collection of large blood volumes. General anesthesia must be administered and the animal placed on its back on a solid surface. The xyphoid process is palpated at the caudal aspect of the animal's sternum. A notch is present on both sides of this process. A 1 inch 22 or higher gauge needle attached to a 1 - 3 mL syringe is inserted into either notch and directed toward the heart as determined by palpating for the heartbeat. Once the needle has been inserted beneath the skin, gentle negative pressure should be applied, by pulling backward on the plunger. The animal must be sacrificed at the completion of the procedure prior to awakening from anesthesia.

**Amputation of the tail tip**

This technique is commonly used in mice, with sample volumes of 0.1–0.2 mL being obtained. Amputation should be restricted to the tail tip (0.5–1 mm should be adequate, and over time only a maximum of 5 mm can be removed). Repeat bleeding is feasible in the short term by removing the clot. Serial amputations resulting in a significant shortening of the tail (>5 mm) are not acceptable. The technique may not be suitable for older animals. Anesthesia is required for any mice 3 weeks old or older.

**Rats**

There are a variety of methods that are utilized to collect blood from rats. The techniques described below are recommended by ACS staff for survival:

- Tail veins
- saphenous vein
- sublingual veins
- orbital venous sinus/plexus is not recommended and requires anesthesia
- terminal (cardiac) blood collection under anesthesia.

**Lateral tail vein venipuncture**

The procedure for collecting blood from the rat's tail vein is similar to the technique described for the mouse. A slightly larger gauge needle (24 gauge) can be utilized. Because of the vein's size, larger blood volumes (approximately 1 mL) may be obtained from adult rats. As for mice, for small blood volume collection, the tail vein can be simply punctured with a small gauge needle. Free flowing drops of blood can be collected, as with the facial or saphenous vein techniques.

**Saphenous vein**

ACS recommends using the saphenous vein (this website is Norwegian but includes generous pictures and an English description).

**Sublingual vein**
This technique is easy to perform in rodents such as rats and is suitable for the removal of large volumes of blood (e.g. 0.2–1 ml) at frequent intervals, limited only by the blood volume to be removed and by the necessary repeated anesthesia. A refined method avoids some of the disadvantages previously seen and can be used for repeated sampling. Rats are anesthetized and held by an assistant in a supine position. The loose skin at the nape of the neck is gathered up in order to produce partial stasis in the venous return from the head. A second person gently pulls out the tongue with a cotton-tipped applicator stick, grasps it with thumb and forefinger and one of the sublingual veins (there is one on each side of the midline) is punctured with a 23–25 g hypodermic needle as near to the tip of the tongue as possible. The rat is turned over to allow blood to drip into a tube and after the requisite volume of blood has been obtained the compression at the scruff of the neck is released and the animal is placed in a supine position. The tongue is again extended in order to stem the flow of blood with a dry cotton-tipped applicator stick; usually there is no need to use any haemostatic agent. With this technique, rats do not show any significant differences in food or water consumption or body weight gain compared with untreated anaesthetized control animals.

Hamsters

There are various acceptable blood collection sites/methods for the hamster. These include the jugular vein, the tail vein, the femoral vein, and the saphenous vein, in addition to those described below.

Orbital venous sinus – this technique is not recommended

The technique describing blood collection from the mouse’s orbital venous sinus should be followed for orbital venous sinus collection in the hamster except the microhematocrit tube should be inserted into the lateral canthus rather than the medial. The technique must be performed under general anesthesia and post-bleeding hemostasis is essential to prevent complications.

Cardiac puncture (Terminal Procedure)

The technique for cardiac puncture from the hamster is identical to that described for the mouse. A 22 gauge needle is recommended. A 3 - 5 cc syringe should be used if large blood volumes are desired. This procedure is performed under general anesthesia as a terminal event only. The animal must be sacrificed at the completion of the procedure prior to awakening from anesthesia.

Guinea Pigs

Acceptable blood collection sites/methods for the guinea pig include the:

- metatarsal vein
- penile vein
- the saphenous vein
- cardiac puncture – terminal only

All are accomplished using the same method as the saphenous vein technique in the mouse. The jugular vein is also an acceptable blood collection site/method but requires anesthesia.

Cardiac puncture (Terminal Procedure)
The technique for cardiac puncture from the guinea pig is identical to that described for mice. This procedure is performed as a terminal event only and general anesthesia is required. The animal must be sacrificed at the completion of the procedure prior to awakening from anesthesia.

Rabbits

Central auricular artery and marginal ear vein (preferred site)

The central auricular artery and the marginal ear vein are useful sites for collection of moderate volumes of blood from rabbits that are to survive the procedure. Vasodilatation can be induced by administering a phenothiazine tranquilizer and alpha adrenergic receptor blocker (acepromazine) at least 5 - 10 minutes, up to one hour, prior to blood collection. A 21G or higher gauge butterfly needle is recommended, but a 1 inch 21G or higher gauge needle and syringe may also be utilized. The insertion site is disinfected using an alcohol soaked pad prior to inserting the needle, bevel up, into the artery or vein. An immediate flash of blood is observed and the blood is allowed to flow out of the open end of the butterfly needle into a suitable container, or blood can be collected directly into a syringe. After the blood collection, it is essential to apply pressure to the artery over the insertion site for at least 3 minutes to provide suitable hemostasis. Significant blood loss can occur from the artery in particular if adequate hemostasis is not provided.

Cardiac puncture (diaphragmatic approach)

Large volumes of blood can be collected directly from the heart of anesthetized rabbits as a terminal event. The technique is similar to that described for mice and rats, however a larger needle and syringe (1.5in >18 gauge ; >20 cc) should be used. Death must be confirmed at the completion of the procedure by administering pentobarbital (120 mg/kg) IV.

Table 1: Approximate blood volumes and recommended maximum blood sample volumes for species of given body weights.

<table>
<thead>
<tr>
<th>Species (weight)</th>
<th>Blood (volume)</th>
<th>7.5% (ml)</th>
<th>10% (ml)</th>
<th>15% (ml)</th>
<th>20% (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse (25 g)</td>
<td>1.8</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Rat (250 g)</td>
<td>16</td>
<td>1.2</td>
<td>1.6</td>
<td>2.4</td>
<td>3.2</td>
</tr>
<tr>
<td>Rabbit (4 kg)</td>
<td>224</td>
<td>17</td>
<td>22</td>
<td>34</td>
<td>45</td>
</tr>
</tbody>
</table>

Table 2: Approximate Blood Sample Volumes Ranges, NIH bleeding guide for mice and rats

<table>
<thead>
<tr>
<th>Body weight (g)</th>
<th>CBV(ml)</th>
<th>1%(ml)</th>
<th>10%(ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>1.10-1.40</td>
<td>.011-.014</td>
<td>.11-.14</td>
</tr>
<tr>
<td>25</td>
<td>1.37-1.75</td>
<td>.014-.018</td>
<td>.14-.18</td>
</tr>
<tr>
<td>30</td>
<td>1.65-2.10</td>
<td>.017-.021</td>
<td>.17-.21</td>
</tr>
<tr>
<td>35</td>
<td>1.93-2.45</td>
<td>.019-.025</td>
<td>.19-.25</td>
</tr>
<tr>
<td>40</td>
<td>2.20-2.80</td>
<td>.022-.028</td>
<td>.22-.28</td>
</tr>
<tr>
<td>125</td>
<td>6.88-8.75</td>
<td>.069-.088</td>
<td>.69-.88</td>
</tr>
</tbody>
</table>
Table 3. Limit volumes and recovery periods

<table>
<thead>
<tr>
<th>Single sampling (e.g. toxicity study)</th>
<th>Approximate recovery period</th>
<th>Multiple sampling (e.g. toxicokinetic study)</th>
<th>Approximate recovery period</th>
</tr>
</thead>
<tbody>
<tr>
<td>% circulatory blood volume removed:</td>
<td></td>
<td>% circulatory blood volume removed:</td>
<td></td>
</tr>
<tr>
<td>7.5%</td>
<td>1 week</td>
<td>7.5%</td>
<td>1 week</td>
</tr>
<tr>
<td>10%</td>
<td>2 weeks</td>
<td>10-15%</td>
<td>2 weeks</td>
</tr>
<tr>
<td>15%</td>
<td>4 weeks</td>
<td>20%</td>
<td>3 weeks</td>
</tr>
</tbody>
</table>

A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes, Diehl, et.al., Journal of Applied Toxicology, 21, 15–23 (2001) [http://www3.interscience.wiley.com/cgi-bin/abstract/76510682/ABSTRACT](http://www3.interscience.wiley.com/cgi-bin/abstract/76510682/ABSTRACT) (click on the full text PDF button to see the article)

Large Animals

The skin over the sampling site may be clipped or shaved to facilitate placement of the needle and the site may be cleaned with disinfectant such as alcohol. It is important that time be taken to locate the vein accurately and that it be distended by gentle pressure before the needle is inserted.

A local anesthetic should be administered carefully between the skin and vein if a large-bore needle (14 gauge or larger bore) is used.

A needle with as large a bore size as possible should be used to ensure efficient blood withdrawal without collapsing the vein, without causing hematoma formation, and without causing blood pressure to drop too rapidly.

Immediately after removal of blood, all animals must have unrestricted access to water.

For adult animals, not more than 15% of the estimated circulating blood volume should be removed in any 4-week period, i.e. 0.9% body weight in cattle and sheep and 1.1% body weight in goats and horses.

Circulating blood volume (litres) can be estimated from body weight (kg) using a conversion factor of 0.06 for cattle and sheep, 0.07 for goats and 0.075 for horses. As a guide, 1% of body weight is the weight of 16 to 17% of the circulating blood volume in sheep and cattle; about 13% in horses and about 14% in goats.

If more than 15% of blood volume is removed, consideration should be given to fluid replacement using lactated Ringer’s solution.
For young animals, the volumes removed should be relatively less. For animals 6 months old and younger, not more than 10% circulating blood volume should be removed.

Hematocrit and hemoglobin as well as body weights must be used to monitor the well-being of animals used to provide relatively large volumes of blood, i.e. 25% of the blood volume in a 4-week period. Individual baseline hematocrit and hemoglobin concentrations must be established for these animals before the start of the process. If the baseline value of any animal does not lie within the normal range, the animal must be evaluated by a veterinarian.

Credits

1) American Association for Laboratory Animal Science
5) University of Washington Training Series
7) Guidelines for the Welfare of Livestock from which Blood is Harvested for Commercial and Research Purposes, Animal Welfare Advisory Committee, Ministry of Agriculture, Wellington New Zealand