



Department of Laboratory Animal Resources

**Guidelines for Blood Collection: Rodents and Rabbits**

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Too much blood collected at any one time may cause hypovolemic shock, physiological stress and even death. If smaller volumes are collected too frequently, anemia may result.

**Collection Volumes**

The total blood volume can be calculated as approximately 6% of normal body weight. The estimated volume at exsanguination is approximately half of the total blood volume.

For multiple blood draws separated by a period of weeks, **a maximum of 1% of the animal's body weight can be removed**, i.e., 0.2 ml from a 20 gram mouse; 4 ml from a 400 gram rat, 40 ml from a 4 kg rabbit. A 14 day recovery period is needed for the average healthy adult animal to recover from this blood loss. Although the blood volume is restored within 24 hours after blood withdrawal, two weeks are needed for all constituents of the blood to return to normal. If less than the maximum amount of blood is withdrawn the animal will replace blood constituents at the rate of 1 ml/kg/day.

By monitoring the hematocrit (packed cell volume=PCV) and/or hemoglobin of the animal, it is possible to evaluate whether the patient has sufficiently recovered from a single blood draw or multiple blood draws. After a sudden or acute blood loss, it takes up to 24 hours for the hematocrit and hemoglobin to reflect this loss. This means that after a 1% of body weight blood loss without fluid replacement, an animal's hematocrit will not show a measurable drop for several hours, and will not stabilize for 24 hours. After 24 hours although the blood volume will normalize, the number of red blood cells (hematocrit) will be measurably reduced. In general, if the animal's hematocrit is less than 35% or hemoglobin concentration is less than 10 g/dl it is not safe to remove the volume of blood listed above.

**Normal Hematocrits of Research Animals**

<b>Dog</b>	29-55	<b>Rabbit</b>	30-50
<b>Cat</b>	25-41	<b>Guinea Pig</b>	37-48
<b>Rhesus</b>	26-48	<b>Hamster</b>	40-61
<b>Sheep</b>	24-45	<b>Rat</b>	36-54
<b>Swine</b>	32-50	<b>Mouse</b>	39-49

Reduce the amount sampled accordingly in obese or compromised animals. For sampling which pushes the upper limits, anesthetic risks rise with the length of anesthesia. Supporting animals with SQ fluids may be a very helpful adjunct to some procedures.

To improve vasodilation effects in rodents, it is helpful to warm the entire patient. This can be accomplished in 10-15 min at 40° C with a special commercially available warming chamber. Care should be taken to prevent overheating. Special instructions are available ahead for rabbits.

When accounting for the amount of blood collected, remember to factor in any extraordinary losses associated with the actual procedure on the individual animal. Regardless of the method of collection used, an animal may not be returned to its cage until complete hemostasis has been achieved

### **Terminal Collections**

Terminal bleeds are only allowed on animals under general anesthesia, and the animal's death must be verified at the end of the bleed. An alternative euthanasia method is recommended after the blood withdrawal.

### **Anesthesia Methods**

The choice of anesthetics is an important consideration when collecting blood from rodents due to physiologic effects of anesthetics. Consult with the attending veterinarian for specific suggestions. Some drugs used in anesthesia/restraint procedures (e.g., xylazine) may promote alterations in normal blood glucose levels. Longer procedures and certain drugs, such as pentobarbital sodium, may result in hypoxia and hypothermia. Blood collection in conjunction with lengthy anesthesia generally contributes to procedural morbidity and mortality.

### **Training Available**

If you are not experienced in blood collection techniques, training is required. If you have questions or comments about any of the techniques presented, contact a DLAR technician. Attention to proper restraint, supportive care, equipment and technical details greatly enhance the success of research requiring blood sampling.

The Department of Laboratory Animal Resources provides blood collection services on a recharge basis if your lab is not staffed to obtain the required specimens. Services are also available to process small volume blood samples from most lab animal species with VetScan/Abaxis analyzers to obtain serum chemistry profiles and hematology data.

## Guidelines for Blood Collection in Mice

Total blood volume = 6% of lean body weight.

Maximum blood collection = 1% of body weight every two (2) weeks.

Example: 25 gm mouse = collect up to 0.25 ml.

<u>Collection Site</u>	<u>Advantages</u>	<u>Disadvantages</u>
<b>Lateral tail vein</b>	<ul style="list-style-type: none"><li>• Anesthesia not required</li><li>• Vein is easily accessed</li></ul>	<ul style="list-style-type: none"><li>• Secure restraint require</li><li>• Yields only small quantities</li><li>• Specialized equipment needed</li></ul>
<b>Orbital Sinus or Plexus</b>	<ul style="list-style-type: none"><li>• Large samples can be collected</li></ul>	<ul style="list-style-type: none"><li>• Anesthesia is required</li><li>• Yields only small quantities</li><li>• Can cause ophthalmic complications</li></ul>
<b>Lateral Saphenous Vein</b>	<ul style="list-style-type: none"><li>• Anesthesia not required</li><li>• Excellent for serial sampling</li><li>• Moderate volume of blood can be collected</li></ul>	<ul style="list-style-type: none"><li>• Requires training</li></ul>
<b>Submandibular or Facial Vein</b>	<ul style="list-style-type: none"><li>• Anesthesia not required</li><li>• Excellent for serial sampling</li><li>• Moderate volumes can be collected</li></ul>	<ul style="list-style-type: none"><li>• Requires training</li><li>• Requires specialized equipment (lancet)</li></ul>
<b>Tail Snip Technique</b>	<ul style="list-style-type: none"><li>• Anesthesia not required</li><li>• Excellent for serial sampling</li></ul>	<ul style="list-style-type: none"><li>• Limited to small sample sizes</li></ul>
<b>Cardiac Puncture (Cardiocentesis)</b>	<ul style="list-style-type: none"><li>• Maximum blood can be collected</li></ul>	<ul style="list-style-type: none"><li>• Requires deep anesthesia</li><li>• Non-survival procedure only</li></ul>

## Guidelines for Blood Collection in Rats

Total blood volume = 6% of lean body weight.

Maximum blood collection = 1% of body weight every two (2) weeks

Example: 250 gm rat = collect up to 2.5 ml.

<u>Collection Site</u>	<u>Advantages</u>	<u>Disadvantages</u>
<b>Lateral tail vein</b>	<ul style="list-style-type: none"><li>• Vein is easily accessed</li><li>• Anesthesia not required</li><li>• Yields moderate quantities</li></ul>	<ul style="list-style-type: none"><li>• Requires special equipment</li><li>• Secure restraint necessary</li></ul>
<b>Ventral Tail Artery</b>	<ul style="list-style-type: none"><li>• Large samples can be collected</li></ul>	<ul style="list-style-type: none"><li>• Anesthesia is required</li><li>• Requires training</li></ul>
<b>Orbital Sinus or Plexus</b>	<ul style="list-style-type: none"><li>• Large samples can be collected</li></ul>	<ul style="list-style-type: none"><li>• Anesthesia is required</li><li>• Requires training</li><li>• Can cause ophthalmic complications</li><li>• Requires post bleed monitoring</li></ul>
<b>Lateral Saphenous Vein</b>	<ul style="list-style-type: none"><li>• Large samples can be collected</li><li>• Anesthesia not required</li><li>• Excellent for serial sampling</li></ul>	<ul style="list-style-type: none"><li>• Requires training</li></ul>
<b>Jugular Vein</b>	<ul style="list-style-type: none"><li>• Large samples can be collected</li></ul>	<ul style="list-style-type: none"><li>• Requires training</li><li>• Anesthesia is required</li></ul>
<b>Anterior Vena</b>	<ul style="list-style-type: none"><li>• Large samples can be collected</li></ul>	<ul style="list-style-type: none"><li>• Requires training</li><li>• Anesthesia is required</li></ul>
<b>Cardiac Puncture</b>	<ul style="list-style-type: none"><li>• Large samples can be collected</li></ul>	<ul style="list-style-type: none"><li>• Requires training</li><li>• Anesthesia is required</li><li>• Non-survival procedure</li></ul>

## Guidelines for Blood Collection in Rabbits

Total blood volume = 6% of lean body weight

Maximum blood collection = 1% of body weight every two (2) weeks

Examples:

2 kg rabbit = 20 ml.

4 kg rabbit = 40 ml

Rabbits may follow the above bleeding schedule as long as packed cell volume (PCV) and total plasma proteins (TPP) are monitored. The occurrence of anemia, hypoproteinemia, or unthriftiness require appropriate supplementation and a rest from further bleeds. The duration of this rest will be determined by the attending veterinarian. Animals should be weighed and logged weekly if on a long term bleeding schedule. Topical irritants, such as xylene, should not be applied to skin for blood collection purposes.

<u>Collection Site</u>	<u>Advantages</u>	<u>Disadvantages</u>
<b>Marginal Ear Vein</b>	<ul style="list-style-type: none"><li>• Anesthesia not required</li><li>• Vein is easily accessed</li></ul>	<ul style="list-style-type: none"><li>• Yields small to moderate</li><li>• Secure restraint required</li><li>• Special equipment recommended</li><li>• Topical anesthetic recommended</li></ul>
<b>Central Ear Artery</b>	<ul style="list-style-type: none"><li>• Large samples can be collected</li></ul>	<ul style="list-style-type: none"><li>• Topical anesthesia is strongly advised (due to the possibility of arterial spasm)</li><li>• Special equipment recommended</li><li>• Training required</li></ul>
<b>Lateral Saphenous Vein</b>	<ul style="list-style-type: none"><li>• Anesthesia not required</li></ul>	<ul style="list-style-type: none"><li>• Training required</li><li>• Special collection set required</li><li>• Small to moderate sample sizes</li></ul>
<b>Cephalic Vein</b>	<ul style="list-style-type: none"><li>• Anesthesia not required</li></ul>	<ul style="list-style-type: none"><li>• Small sample sizes</li><li>• Training recommended</li><li>• Some specialized equipment needed</li></ul>
<b>Jugular Vein</b>	<ul style="list-style-type: none"><li>• Large samples can be collected</li></ul>	<ul style="list-style-type: none"><li>• Anesthesia is required</li><li>• Training required</li><li>• Requires more skill</li></ul>
<b>Anterior Vena Cava</b>	<ul style="list-style-type: none"><li>• Large samples can be collected</li></ul>	<ul style="list-style-type: none"><li>• Anesthesia is required</li><li>• Requires more skill</li><li>• Risk of cardiac tamponade</li></ul>
<b>Cardiac Puncture</b>	<ul style="list-style-type: none"><li>• Maximum quantity of blood</li></ul>	<ul style="list-style-type: none"><li>• Requires deep anesthesia</li><li>• Non-survival procedure only</li></ul>

#### Technique Example: Marginal Ear Vein:

Place the rabbit in a rabbit restrainer. Using clippers, remove the hair from the ear. Place a finger, loosely fitting paper clip, or other form of light tourniquet at the base of the ear. Use a 25-gauge, 5/8-inch or 22-gauge, 1-inch needle. Small quantities can be collected from the hub of a 25-gauge needle directly into a microhematocrit tube. The larger needle is attached to a syringe and blood is collected slowly into the syringe to avoid collapsing the vein. If blood does not flow readily, there may be a clot formation in the needle or too much negative pressure applied to the syringe. Release the negative pressure and slowly rotate the needle. Repeat these steps as needed until the desired quantity of blood has been collected.

A tranquilizer, such as Innovar-Vet or Ace-Promazine, works very well as a vasodilator as well as providing a tranquilizing effect. Administer Innovar-Vet at a dose of 0.1 to 0.2 ml per rabbit SQ or IM; or Ace-Promazine 0.25 to 0.5 mg/kg SQ or IM. The best results are obtained when administered 10-15 minutes prior to blood collection.

#### Technique Example: Central Artery

Place the rabbit in a rabbit restrainer. Using clippers, remove the hair from the ear. A 1 to 1-1/2-inch needle ranging in size from 22-gauge to 20-gauge is recommended for blood collection. Some investigators prefer to break off the hub of the needle and use the needle shaft for collection or to use a vacutainer needle. Extend the ear away from the rabbit's head to provide a flat surface and insert the needle into the artery. Once blood begins to flow, lower the ear and position the receptacle under the needle for blood collection. Occasionally, the artery may constrict during the collection process, shutting off the flow of blood. Hold the needle securely in place until the rabbit relaxes, usually within one minute. Gentle massage of the artery at the base of the ear may enhance blood flow. Clot formation in the needle can also prevent blood flow. If no blood flows, remove the needle and repeat the procedure using a new needle. Insert the new needle into the artery proximal to the previous site (closer to the base of the ear).

Some experienced operators find it helpful to handle the rabbit without undue stress and to provide supplemental heat to the ear from a 100 watt light bulb. Both practices will help to dilate the vessels.

#### Technique Example: Saphenous Vein

An attendant restrains the rabbit, exposing the rear leg to the person drawing the specimen. Hair is clipped from the lateral saphenous vein on the lower rear limb. The vein is occluded by the attendant and the phlebotomist cleans and dries the skin over the vein with alcohol and sterile gauze. Using a scalp vein set primed with sodium heparin, a 21-24 gauge needle is inserted into the occluded vessel and blood is aspirated into the connected 3 ml syringe prefilled with 1 ml of air. The syringe may be gently rocked while the blood is collected to avoid clotting. Pressure is placed on the venipuncture site for about 20 seconds after withdrawing the needle.

## **Retro Orbital Bleeding in Rodents**

Procedure and responsibilities for monitoring animals post bleeding.

### **Research Staff**

1. Inform DLAR management of the upcoming bleeding schedule preferably >2 days prior to include the approximate number of cages.
2. Each cage must have an observation card supplied by DLAR. The individual performing the procedure should complete this card with date, their signature and any comment or concerns pertaining to the animals in the cage, followed by placing the card on the cage behind the cage card in the vertical position.
3. For 1-2 days post bleeding research staff are responsible for checking the animals.
  - Any concerns must be reported directly to DLAR veterinarian or management staff.
  - All animal deaths must be accounted for, reported and saved for the veterinarian to necropsy. The Vet Care Card procedure must be followed for saving and identifying all mortalities.

### **DLAR Staff:**

1. The DLAR manager will inform the veterinarian of the bleeding schedule.
2. Will supply the observation cards for identifying each cage.
3. For 1-3 days post bleeding depending on condition, the DLAR technician assigned to the animal room will complete the daily observation and complete the observation card.
  - Any concern will be reported to the veterinarian.
  - Observation cards will be collected and filed with the veterinarian post observation time.

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