

# BLOOD COLLECTION TECHNIQUES AND LIMITS

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## Table of Contents

1. [Purpose & Responsibilities](#)
  2. [General Procedures](#)
    - a. Rodent (Mouse and Rat) Procedures
    - b. Rabbit and Guinea Pig Procedures
  3. [Training](#)
  4. [Blood Collection Limits](#)
  5. [References](#)
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## 1. Purpose

This document outlines the preferred methods for collecting blood from animals and blood collection volume and frequency limits. Blood collection for experimental purposes must comply with the investigator's Animal Care and Use Committee (ACUC) approved protocol, including approved collection techniques, volumes, and frequencies. The Office of Laboratory Animal Care (OLAC) veterinary staff trains investigators in various collection techniques. The veterinary staff may collect blood for veterinary care purposes using accepted clinical techniques ensuring volumes collected do not adversely affect animal health.

## 2. General Procedures

New, sterile needles must be used for each venipuncture. Do not reuse needles between animals or between collection episodes.

Electric heat pads/lamps should not be used for vasodilation. Please consult with OLAC veterinary staff or training coordinator for safe and effective vasodilation methods.

Amputating digits for the collection of blood is incompatible with the humane care and use of laboratory animals and is not permitted.

Requests to use alternative blood collection methods not listed in Table 1, including transection of vessels or tail clipping, may be considered following consultation with an OLAC veterinarian. The alternate method must be approved in the ACUC approved protocol. Survival blood collections may be performed as outlined in Table 1 (*see next page*).

Non-survival exsanguinations require administration of surgical levels of anesthetic or lethal doses of euthanasia agents prior to collection (injectable agent or carbon dioxide [CO<sub>2</sub>]). Survival blood collection from the heart must be conducted under general anesthesia and is not permitted without scientific

justification and competency certification by the OLAC veterinary staff or training coordinator prior to performing the technique independently.

Table 1.

Species	Recommended site for blood collection
Mouse*	Tail vein or artery, lateral saphenous vein, facial vein Retroorbital sinus subject to stipulations outlined in this guideline and in the animal use protocol.
Rat*	Tail vein or artery, saphenous vein, lateral saphenous vein, jugular vein Retroorbital sinus subject to stipulations outlined in this guideline and in the animal use protocol.
Rabbit*	Marginal ear vein (small volumes), auricular artery (large volumes)
Guinea pig*	Ear vein, saphenous vein Anterior vena cava collection subject to stipulations outlined in this guideline and in the animal use protocol.
*Cardiac collection subject to stipulations outlined in this guideline and in the animal use	

#### a. Rodents (Mouse and Rat)

- i. Preferred sites for blood collection from mice and rats include tail veins, saphenous veins (hind limb), or, with proper training, the facial vein and jugular vein (competency certification by OLAC required prior to performing this technique independently). These techniques may be performed in conscious animals. Use of the saphenous vein requires clipping the fur from the area around the vein and swabbing with 70% alcohol prior to puncture.
- ii. Retro-orbital blood collection must be performed under general anesthesia.
  1. Survival retro-orbital blood collection is discouraged but may be approved with robust scientific justification. When approved, survival retro-orbital blood collection must include the administration of an antibiotic ophthalmic and topical anesthetic to both eyes to minimize pain and distress and prevent desiccation and infection. Individuals performing retro-orbital blood collection must be adequately trained and certified for competency by OLAC prior to performing this technique independently due to the potential for significant complications.
  2. Sufficient time must be provided to allow the eye to heal before it is re-used for blood collection. This generally requires 4 weeks for recovery to minimize pathology in orbital tissues, including hemorrhage, inflammation, and infection.
  3. If this technique must be performed more than once on one animal, alternating eyes at each blood collection is required.

## **b. Rabbits and Guinea Pigs**

- i. The ear veins are often the preferred sites for blood collection from rabbits. Rabbits become sensitive to ear manipulation and can become resistant to repeated ear venipuncture. Ear venipuncture may be performed in conscious animals but use of topical anesthetics, such as EMLA cream, may reduce this resistance and promote animal welfare. Use of the auricular (ear) artery may be necessary for collecting larger volumes. Use of the ear vein may require clipping the fur from the area around the vein and swabbing with 70% alcohol prior to puncture.
- ii. Marginal ear veins may be available for collecting small quantities of blood from guinea pigs. The ear must be swabbed with 70% alcohol prior to puncture.
- iii. Cardiac puncture for blood collection requires general anesthesia, as does collection of blood from the guinea pig anterior vena cava. Experimental survival cardiac or vena cava blood collection requires scientific justification and approval in the animal use protocol.

## **3. Training**

OLAC offers training for blood collection in a variety of laboratory species. In addition, OLAC provides blood collection services for investigators for a nominal fee. Retro-orbital and jugular vein, as well as survival intracardiac blood collection requires competency certification by the OLAC veterinary staff or training coordinator prior to performing these techniques independently.

Please refer to ACUC pre-approved verbiage for Blood Collection Procedures for further details regarding blood collection procedures in select species.

## **4. Blood Collection Limits**

The ACUC limits one time survival blood collection to 15% of an animal's blood volume in most circumstances. Serial blood sampling limit vary by species, strain, and frequency of blood collection as outlined in Tables 2 and 3. The ACUC may require monitoring for anemia (using assays such as hematocrit and/or serum protein levels) when repeated collections or collection of larger volumes are required. Blood collected for diagnostics or other veterinary procedures must be considered when evaluating total volume available for experimental use. In all cases blood collection volumes should be limited to the minimum volume that will allow for successful experimentation or diagnostics.

*(See Tables 2 & 3 on next page)*

**Table 2.** (Source: Fox et al. 2002; Diehl et al 2001)

Species	Blood Volume Mean (ml/kg)	Blood Volume Range (ml/kg)	Blood Volume (average)		
			7.5%	10%	15%
Mouse (25 g average weight)	58.6	55-80	110µl	146µl	220µl
Rat (250 g)	64	58-70	1.2 ml	1.6 ml	2.4 ml
Rabbit (4 kg)	56	44-70	17 ml	22 ml	34 ml
Nonhuman primate (NHP; 8 kg)	56	55-75	34 ml	45 ml	67 ml

**Table 3.** (Source: Diehl et al., 2001)

Single sampling		Multiple sampling	
% Circulatory blood volume removed	Approximate recovery period	% Circulatory blood volume removed (cumulative volume)	Approximate recovery period
7.5%	1 week	7.5%	1 week
10%	2 weeks	10-15%	2 weeks
10-15%	4 weeks	20%	3 weeks

## 5. References

- Diehl, K.H., Hull, R., Morton, D., Pfister, R., Rabemampianina, Y., Smith, D., Vidal, J.M., & van de Vorstenbosch, C. (2001). A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes. *Journal of Applied Toxicology*, 21, 15–23.
- Fox, J.G., Anderson, L.C., Loew, F.M., Quimby, F.W. (Eds.). (2002). *Laboratory Animal Medicine* (2<sup>nd</sup> ed.). New York City, NY: Academic Press
- Hem, A., Smith, AJ, & Solberg, P. (1998). Saphenous Vein Puncture for Blood Sampling of the Mouse, Rat, Hamster, Gerbil, Guinea Pig, Ferret, and Mink. *Laboratory Animals*, 32, 364-68