

Guidelines for Survival Blood Collection in Mice and Rats

Overview

These guidelines have been developed to assist investigators and National Institutes of Health (NIH) Institute/Center (IC) Animal Care and Use Committees (ACUC) in their choice and application of survival rodent bleeding techniques. The guidelines are based on peer-reviewed publications¹⁻⁹ and data and experience accumulated at NIH. The researcher and the veterinary staff should decide which survival bleeding technique is appropriate. All blood sampling (including technique, frequency, and volume) must be in an approved Animal Study Proposal (ASP) or referred to in an ACUC reviewed Standard Operating Procedure (SOP). It is the responsibility of both the researcher and the IC ACUC to select/approve the procedures that result in the least pain and distress to the animal, while adequately addressing the needs of the experimental design. Any exceptions to these guidelines, e.g., increase in blood volume or frequency to be collected, retro-orbital bleeding without use of topical anesthesia, or surgical cannulation must be scientifically justified in the ASP.

General

As with any procedure, training is critically important. **Training and experience of the phlebotomist in the chosen procedure are of paramount importance. Training opportunities and resources, including access to experienced investigators and veterinarians, must be made available to new personnel.** Each Principal Investigator must ensure sufficient training for individuals performing these technical procedures. In addition, individual IC ACUCs should establish lines of accountability to oversee the training of their personnel. The procedures utilized must be reviewed and approved by the IC ACUC prior to implementation. The Office of Animal Care and Use (OACU) has additional training resources on its website to include survival rodent blood collection: <https://oacu.oir.nih.gov/training-resources> Factors to consider when selecting the appropriate blood collection technique for research purposes include, but are not limited to:

- The species to be bled.
- The size and age of the animal to be bled and the estimated total blood volume.
- The type of the sample required (e.g., serum, whole blood cells, etc.).
- The quality of the sample required (e.g., sterility, tissue fluid contamination, etc.).
- Appropriate anticoagulant for the type of assay.
- The quantity of blood required, considering extraneous blood loss due to a selected method.
- The frequency of sampling.
- The health status of the animal being bled (e.g., hydration status).
- The training and experience of the phlebotomist.
- The size and type of capillary tube, lancet or needle is appropriate.

The effect of the site, restraint or anesthesia on the blood parameter measured¹⁰⁻¹⁵ The acceptable quantity and frequency of blood sampling is dependent on the circulating blood volume of the animal and the red blood cell (RBC) turnover rate (RBC life span of the mouse: 38-47 days. RBC life span of the rat: 42-65 days.¹⁹⁻²¹). The approximate circulating blood volume (CBV or total blood volume TBV) of adult rodents varies with species and body weight [mouse 63 to 80 ml/kg (mean 72 ml/kg) and rat 58-70 ml/kg (mean 64 ml/kg)].³ Of the circulating blood volume, approximately 10% of the total volume can be safely removed every 2 to 4 weeks, 7.5% every 7 days, and 1% every 24 hours.^{17,18}

Based on animal welfare indices the NIH veterinary recommended blood volume to use is 55 to 70 ml/kg when calculating quantity. Volumes greater than recommended should be justified in the ASP and

appropriate fluid and/or cellular replacement provided. Calculated blood sample ranges, based on recommended body weight are provided in Table 1.

Species	Body weight (g)	*CBV(ml)	~1% CBV every 24 hrs†	~7.5% CBV every 7 days†	~10% CBV every 2 - 4wks†
Mouse	20	1.10 - 1.40	11 - 14 µl	90 - 105 µl	110 - 140 µl
	25	1.37 - 1.75	14 - 18 µl	102 - 131 µl	140 - 180 µl
	30	1.65 - 2.10	17 - 21 µl	124 - 158 µl	170 - 210 µl
	35	1.93 - 2.45	19 - 25 µl	145 - 184 µl	190 - 250 µl
	40	2.20 - 2.80	22 - 28 µl	165 - 210 µl	220 - 280 µl
Rat	125	6.88 - 8.75	69 - 88 µl	516 - 656µl	690 - 880 µl
	150	8.25 - 10.50	82 - 105 µl	619 - 788 µl	820 - 1000 µl
	200	11.00 - 14.00	110 - 140 µl	825 - 1050 µl	1.1 - 1.4 ml
	250	13.75 - 17.50	138 - 175 µl	1.0 - 1.3 ml	1.4 - 1.8 ml
	300	16.50 - 21.00	165 - 210 µl	1.2 - 1.6 ml	1.7 - 2.1 ml
	350	19.25 - 24.50	193 - 245 µl	1.4 - 1.8 ml	1.9 - 2.5 ml
	*Circulating blood volume (1ml = 1000µl)		†Maximum sample volume for that sampling frequency		

The following guidelines refer to the most frequently used survival sampling sites: a) submandibular vein; b) saphenous vein; c) tail vein; d) retro-orbital. Blood withdrawal by cardiac puncture is considered a euthanasia procedure and should be performed only after ensuring that the animal is under deep anesthesia, as evidenced by lack of response to a painful stimulus (e.g., toe or tail pinch).

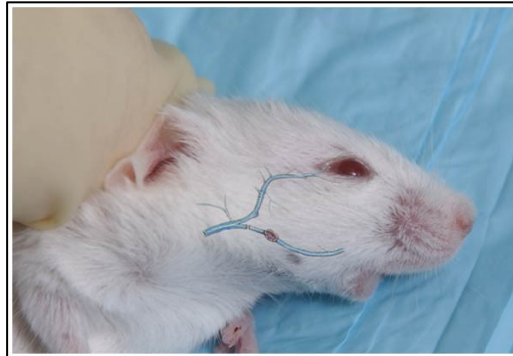
Procedures

Basic recommendations for each survival bleeding technique are provided below.²⁶

Submandibular (facial vein) Blood Sampling (limited to adult mice):^{8,10-12,22-24}

- Obtainable blood volumes: medium to large. This technique is suitable for collecting up to the maximum allowable volume for the given weight and sampling interval (see Table 1).
- Repeated sampling is possible by alternating sides of the face.
- General anesthesia not required.
- Sample may be a mixture of venous and arterial blood.
- Can be performed rapidly and with a minimal amount of equipment, allowing for rapid completion.
- Sample volume can be partially controlled with the size of needle (20 gauge or smaller) or lancet (4 mm) used to puncture the site.
- Proper manual restraint of awake animals results in proper site alignment and venous compression for good blood flow.
- Blood is obtained from a small vascular bundle at the back of the jaw. The puncture site is caudal to the small cowlick.

- Recommended for a single draw per side no more often than once per week.
- Clinical chemistry values may be higher with this method than with the retro-orbital plex route.¹⁴



Lateral Tail Vein or Ventral artery Sampling:²⁹⁻³¹

- Obtainable volumes by needle nick or cannulation: artery – medium to large. Vein – small
- In general, arterial sampling produces larger volumes and is faster, but special care must be taken to ensure adequate hemostasis. For this reason, the artery should only be used if large volumes are needed.
- Can be used in both rats and mice by cannulating the blood vessel with a needle, or by superficially nicking the vessel perpendicular to the tail.
- General anesthesia not required, although effective restraint is required.
- Sample collection by nicking the vessel is easily performed in both species but produces a sample of variable quality that may be contaminated with tissue products. Sample quality decreases with prolonged bleeding times and “milking” of the tail. Sample collection using a needle minimizes contamination of the sample but is more difficult to perform in the mouse.
- Repeated collections possible. With tail nicking, the clot/scab can be gently removed for repeated small samples if serial testing is required (e.g., glucose measures, etc.).
- In most cases warming the tail with the aid of a circulating warm water or warm compresses will increase obtainable blood volume. Caution: never immerse tail in hot water!

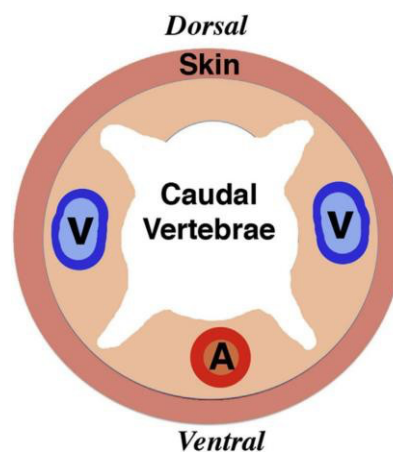


Figure 1. Cross-section of rodent tail, showing vessels used for blood collection.⁷

Tail Clip Sampling:³¹

- Obtainable volume: small
- Can be used in both rats and mice by clipping (e.g., amputating) no more than 1mm of the distal tail in mice or 2 mm in rats.
- Produces a sample of variable quality that may be contaminated with tissue products.
- Sample quality decreases with prolonged bleeding times and “milking” of the tail.
- Repeated collections possible. The clot/scab can be gently removed for repeated small samples if serial testing is required (e.g., glucose measures, etc.).
- In most cases warming the tail with the aid of a heat lamp or warm compresses will increase obtainable blood volume.
- When performing tail clipping, consideration should be given to anesthesia/analgesia, particularly if the tail has been previously clipped for genotyping. If a topical hypothermic anesthetic is used, blood will flow as the tail re-warms. If a local anesthetic is applied, adequate contact time should be allowed for it to take effect.

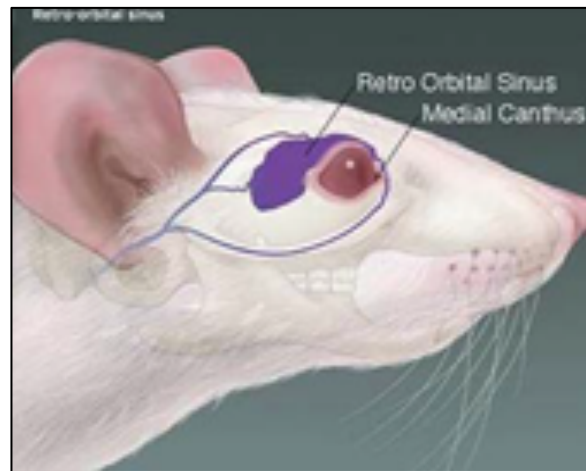
Saphenous Sampling (medial or lateral approach):²⁶⁻²⁸

- Obtainable blood volumes: small to medium.
- Can be used in both rats and mice by piercing the saphenous vein with a needle.
- Hair may be removed from the site to aid in visualization of the vessel.
- Recommend applying petroleum jelly over the vessel prior to needle stick to prevent blood wicking onto the fur, and in turn enhancing the total blood volume collected.
- Variable sample quality
- General anesthesia is not required, although effective restraint is required¹⁷
- Requires more hands-on training than tail or retro-orbital sampling to reliably withdraw more than a minimal amount of blood.
- Although more esthetically acceptable than retro-orbital sampling, prolonged restraint and site preparation time can result in increased animal distress when handling an awake animal.
- Temporary favoring of limb may be noted following the procedure.
- The clot/scab can be gently removed for repeated small samples if serial collection is required.

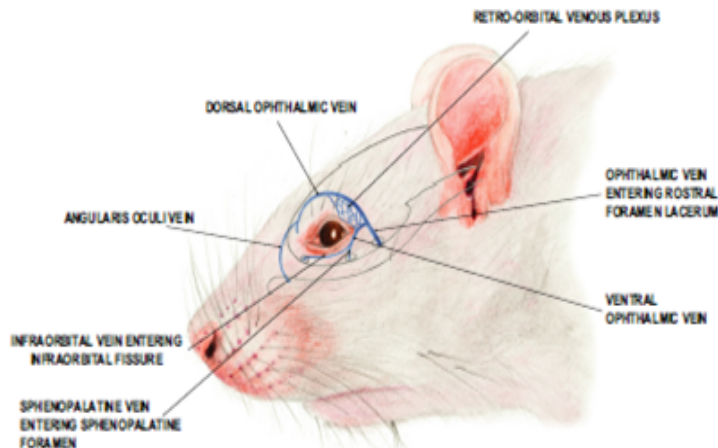
Retro-orbital Sinus/Plexus Sampling:^{20,21,22}

- Obtainable blood volumes: medium to large. This technique is a suitable for collecting up to the maximum allowable volume for the given weight and sampling interval (see Table 1).
- Rapid – large number of animals can be bled within a short period of time.
- Retro-orbital sampling can be used in both mice and rats by penetrating the retro-orbital sinus in mice or plexus in rats with a sterile hematocrit capillary tube or Pasteur pipette (carefully monitor volume of blood drawn—capillary tubes vary widely in size). Sterile tubes are recommended to help avoid periorbital infection and potential long-term damage to the eye.
- In rats, the presence of a venous plexus rather than a sinus can lead to greater orbital tissue damage than in the mouse. General anesthesia must be used unless scientific justification is provided and approved by the IC ACUC. In addition, a topical ophthalmic anesthetic, e.g., proparacaine or tetracaine drops, is recommended prior to the procedure, and may be considered an analgesic. Due to the anatomy of the rat retro-orbital plexus, ARAC believes that retro-orbital bleeding performed in rats by a trained practitioner represents more than “minimal or transient pain and distress” and therefore should be considered a USDA Column D procedure.
- Insert tube at the medial canthus under the nictitating membrane at a 45-degree angle, aiming for the back of the orbit.

- Good sample quality. Potential contamination with topical anesthetic, if used, should be taken into account.
- A minimum of 10 days should be allowed for tissue repair before repeat sampling from the same orbit, otherwise, the healing process may interfere with blood flow.
- Alternating orbits should not be attempted until the phlebotomist is proficient in obtaining samples from the orbit accessed most readily by the dominant hand i.e., a right-handed individual should gain proficiency withdrawing samples from the right orbit.
- In the hands of an unskilled phlebotomist, retro-orbital sampling has a greater potential than other blood collection routes to result in complications. When personnel are undergoing training in retro-orbital blood collections, general anesthesia for the animals is required, and the animals are euthanized immediately following procedure.
- In mice, general anesthesia is recommended if compatible with experimental design. If retro-orbital bleeding is conducted without general anesthesia, a topical ophthalmic anesthetic e.g., proparacaine or tetracaine drops, must be applied prior to the procedure.
- In both mice and rats, care must be taken to ensure adequate hemostasis following the procedure.
- Protect the contralateral eye (not being bled) by instilling ophthalmic lubricant and avoiding pressure damage.



Mouse Retro Orbital Sinus



Rat Retro Orbital Plexus

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