# 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. Terminal bleeding techniques (e.g., cardiac puncture, portal vein, vena cava) are not covered in this guideline. The guidelines are based on peer-reviewed publications(1-11) 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 described in an approved Animal Study Proposal (ASP) or referred to in an ACUC-approved 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 (PI) 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 collection, including extraneous blood loss due to a selected method.
- The frequency of blood collection.
- 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.
- Blood parameter measurements are impacted by blood sampling techniques (12-20).

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 (21)
- RBC life span of the rat: 42-65 days) (22, 23).

The approximate circulating blood volume (CBV or total blood volume TBV) of adult mice/rats varies with species and body weight:

- Mouse 63 to 80 ml/kg (mean 72 ml/kg)
- Rat 58-70 ml/kg (mean 64 ml/kg)].[3]

**To decrease the risk of hypovolemic shock, NIH veterinarians recommend reducing the circulating blood volume used for calculations by 8%: Mouse 58-74 ml/kg (mean 66 ml/kg); Rat 53 to 64 ml/kg (mean 59 ml/kg).**(24) 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 (9, 22, 23, 25). Note, most animals go into shock with 25%-30% acute blood loss occurs and blood loss of greater than 30% can result in death.(24, 26)

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 and Table 2.

**Table 1: Maximum Blood Sample Volumes for Mouse by Body Weight**

| Species | Body weight (g) | Circulating Blood Volume 1ml = 1000µl | Maximum volume for 24-hour serial collection (1%) | Maximum volume for 7-day serial collection (7.5%) | Maximum volume for serial collection at 14-28d interval (10%) |
|---|---|---|---|---|---|
| Mouse | 20 | 1160 - 1480 µl | 11 - 14 µl | 87 - 111 µl | 116 - 148 µl |
| | 25 | 1450 - 1850 µl | 14 - 18 µl | 108 - 138 µl | 145 - 185 µl |
| | 30 | 1740 - 2219 µl | 17 - 22 µl | 130 - 166 µl | 174 - 221 µl |
| | 35 | 2030 - 2590 µl | 20 - 25 µl | 152 - 194 µl | 203 - 259 µl |
| | 40 | 2320 - 2960 µl | 23 - 29 µl | 174 - 222 µl | 232 - 296 µl |

**Table 2: Maximum Blood Sample Volumes for Rat by Body Weight**

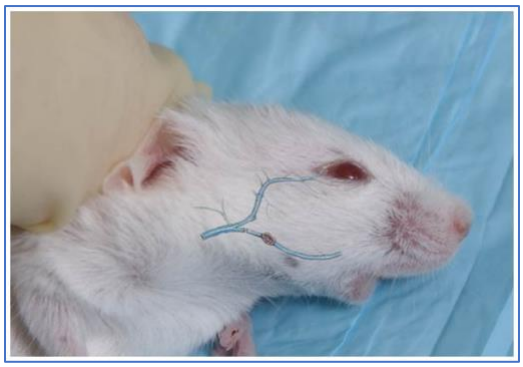
| Species | Body weight (g) | Circulating Blood Volume (ml) 1ml = 1000µl | Maximum volume for 24-hour serial collection (1%) | Maximum volume for 7-day serial collection (7.5%) | Maximum volume for serial collection at 14-28d interval (10%) |
|---|---|---|---|---|---|
| Rat | 125 | 6.62 - 8.00 ml | 0.07 - 0.08 ml | 0.50 - 0.60 ml | 0.66 - 0.80 ml |
| | 150 | 7.95 - 9.60 ml | 0.08 - 0.10 ml | 0.60 - 0.72 ml | 0.79 - 0.96 ml |
| | 200 | 10.60 - 12.80 ml | 0.11 - 0.13 ml | 0.80 - 0.96 ml | 1.06 - 1.28 ml |
| | 250 | 13.25 - 16.00 ml | 0.13 - 0.16 ml | 0.99 - 1.20 ml | 1.33 - 1.60 ml |
| | 300 | 15.90 - 19.20 ml | 0.16 - 0.19 ml | 1.19 - 1.44 ml | 1.59 - 1.92 ml |
| | 350 | 18.55 - 22.40 ml | 0.19 - 0.22 ml | 1.39 - 1.68 ml | 1.85 - 2.24 ml |

**Procedures:**

Basic recommendations for common survival bleeding technique are provided below (27, 28).

Submandibular (facial vein) Sampling (*limited to adult mice*) (12-14, 29-31):

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



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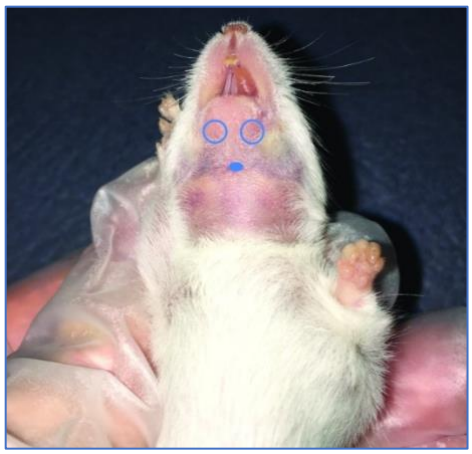
Submental Sampling (limited to adult mice) (19, 32, 33):

- 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).
- Recommended for a single draw per side no more often than once per week.
- General anesthesia is not required.
- The submental route targets the inferior labial and facial veins located under the chin.
- Proper manual restraint of awake animals requires gripping the scruff near the ears so that the head is immobilized and tilted back to expose the submental region.
- The target sites are visibly darkened areas located craniolateral to the fur whorl on the ventral chin midline. A 4-5 mm lancet can be used to pierce the area.
- There were no significant differences in clinical chemistry values when comparing submandibular and submental collection sites (19).



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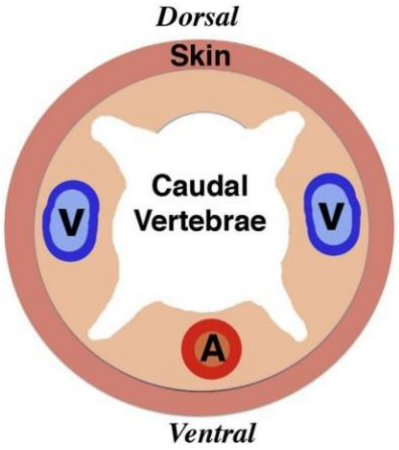
Lateral Tail Vein, Tail Nick, or Ventral artery Sampling (34-36):

- 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!

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Tail Clip Sampling (36):

- 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.

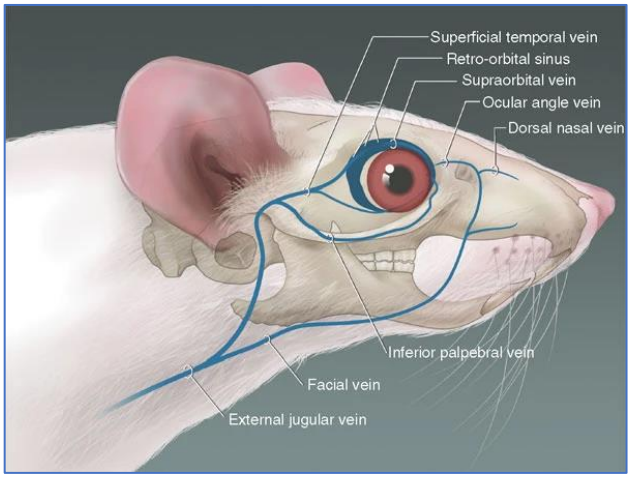
Saphenous Sampling (medial or lateral approach) (29, 37, 38):

- 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 required (25).
- 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 (21, 22, 31, 39, 40):

- 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.

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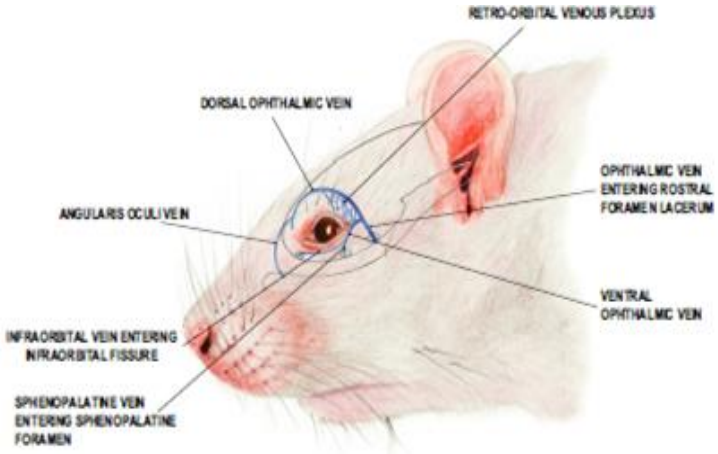


Rat Retro Orbital Plexus

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