Guidelines for Blood Collection in Mice and Rats

This document provides direction and guidance on appropriate blood collection methods and volumes for animals used in research at the AECOM. These guidelines (adapted from the NIH ARAC Guidelines) are intended for use by qualified personnel performing blood collection as described in an IACUC-approved Animal Protocol.

All blood sampling (including technique, frequency and volume) must be in an IACUC-approved Animal Protocol or referred to in an ACUC reviewed Standard Operating Procedure. It is the responsibility of both the researcher and the IACUC 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 IACUC-approved Animal Protocol.

General: As with any procedure, training is critically important. Training and experience of the phlebotomist in the chosen procedure are of paramount importance.

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 (sterility, tissue fluid contamination, etc.)
- The quantity of blood required (taking into account extraneous blood loss due to a selected method)
- The frequency of sampling
- The health status of the animal being bled
- The training and experience of the phlebotomist
- The size and type of capillary tube is appropriate
- The effect of the site, restraint or anesthesia on the blood parameter measured[10-15]

The limitations for blood collection preserve the health status of the animal and maintain the validity of experimental results based on blood samples. The guidelines provided are for healthy, normal adult animals. Animals that are young, aged, stressed, have undergone experimental manipulations, or are suffering from cardiac or respiratory disease may not be able to tolerate this amount of blood loss.

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.[‡]

The approximate circulating blood volume 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 recommended blood volume to use is 55 to 70 ml/kg when calculating quantity. Volumes greater than recommended must be justified in IACUC-approved Animal Protocol and appropriate fluid and/or cellular replacement provided. **Calculated blood sample ranges, based on recommended body weight are provided in Table 1.**

[‡]RBC life span of the mouse: 38-47 days. RBC life span of the rat: 42-65 days.[19-21]

Table 1: Calculated Blood Sample Volumes for Species and Range of Body Weights					
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 plexus; b) saphenous vein); c) tail vein; d) retro-orbital; e) submental. **Blood withdrawal by cardiac puncture is considered an 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.

Submandibular Blood Sampling (limited to adult mice):[8,10-12,22-24]

- Obtainable blood volumes: medium to large.
- 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 drawn from a small vascular bundle at the back of the jaw. The puncture site is caudal to the small cowlick
- Not recommended for serial draws (> than 2 draws per side)[25]
- Clinical chemistry values may be higher with this method than with the retro-orbital plexus route.[14]

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.
- Variable sample quality
- General anesthesia is not required, although effective restraint is required. [17]
- Requires more hands-on training than tail or retro-orbital sampling to reliably withdraw more than a

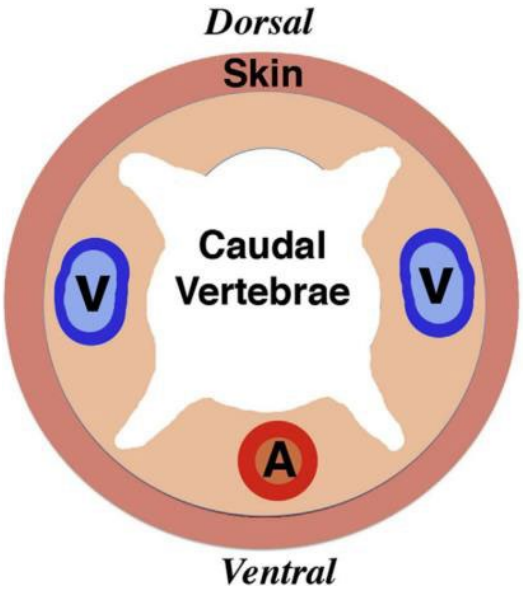
minimal amount of blood.

- 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.
- Application of sterile petroleum jelly to the site may assist the blood to bead and in turn enhance total blood volumes captured.
- The clot/scab can be gently removed for repeated small samples if serial collection is required.

Lateral Tail Vein or Ventral/Dorsal Artery Sampling:[29-31]

- Obtainable volumes for cannulation or nicking: 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 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 bleedingtimes and "milking" of the tail.
- Sample collection using a needle (cannulation) 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.

Figure 1. Cross-section of rodent tail, showing vessels used for blood collection.[6]



Retro-orbital Sinus/Plexus Sampling: [20,21,22,23]

- Obtainable volume: medium to large.
- 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. Sterile tubes are recommended to help avoid periorbital infection and potential long-term damage to the eye.
- 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 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 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 IACUC. 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, NIH 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.
- In both mice and rats, care must be taken to ensure adequate hemostasis following the procedure.

Tail Clip Sampling:[33]

- **Discouraged** except in cases where repeated collections are needed. The clot/scab can be gently removed for repeated small samples if serial testing is required (e.g., glucose measures, etc.).
- When performing tail clipping, general anesthesia and cautery is required if >21 to 35 days old. If mice are >35 days old, this is considered a surgical biopsy procedure requiring general anesthesia, pre-emptive analgesia lasting 24 hours, and cautery for hemostasis. *Give consideration to rodents where 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 must be allowed for it to take effect.
- 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.
- In most cases warming the tail with the aid of a heat lamp or warm compresses will increase obtainable blood volume.

Submental Sampling (adult mice):[32,33]

- Obtainable blood volumes: medium to large
- Easy to perform
- Results in high quality samples
- Collect under anesthesia to facilitate the procedure

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