Guidelines for Survival Bleeding of Mice and Rats

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 publications[1,2,3,4,5,6,7,8] as well as on data and experience accumulated at NIH. The Investigator and veterinary staff should decide which method of blood withdrawal to use. As with any procedure, training is critically important. The comfort and level of skill of an investigator with a procedure, as well as the sample volume and frequency of sampling should be considered when choosing a method. It is the responsibility of both the investigator and IC ACUC to ensure use of techniques and procedures which 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 to be collected or retro-orbital bleeding without use of anesthesia as outlined below, must be described in IC ACUC guidelines or must be scientifically justified in the Animal Study Proposal (ASP) and approved by the IC ACUC.

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 IC ACUC should establish lines of accountability to oversee the training of its personnel. The procedures utilized must be reviewed and approved by the IC ACUC prior to their implementation.

Factors to consider in choosing the blood withdrawal technique appropriate for the purpose at hand include, but are not limited to:

- The species to be bled.
- The size 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.
- The frequency of sampling.
- The health status of the animal being bled.
- The training and experience of the phlebotomist.
- The effect of the site, restraint or anesthesia on the blood parameter measured.[9,10,11,12,13,14]

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.[ǂ] The approximate circulating blood volume of rodents is 55 to 70 ml/kg of body weight. 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.[15,16]

Volumes greater than recommended should be justified in the ASP and appropriate fluid and/or cellular replacement provided. Blood sample ranges, based on body weight are provided in Table 1.

[ǂ] RBC life span of the mouse: 38-47 days. RBC life span of the rat: 42-65 days.[17,18,19]

Table 1: Approximate Blood Sample Volumes for a Range of Body Weights				
Body weight (g)	*CBV(ml)	1% CBV *every 24 hrs†*	7.5% CBV *every 7 days†*	10% CBV *every 2 - 4wks†*
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	12 - 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
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 - 2450 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) retro-orbital; b) facial vein; c) saphenous vein; d) tail vein; and e) jugular vein. 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). A list of the issues that should guide the choice of survival blood collection route(s) is noted below, and an abbreviated summary is provided in Table 2.

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

- 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 capillary tube or Pasteur pipette.
- Rapid – large number of animals can be bled within a short period of time.
- Obtainable volume: medium to large.
- 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 before attempting to obtain samples from the left orbit.
- In the hands of an unskilled phlebotomist, retro-orbital sampling has a greater potential than other blood collection routes to result in complications.
- 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 IC ACUC. In addition, a topical ophthalmic anesthetic, e.g. proparacaine or tetracaine drops, is recommended prior to the procedure. 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.

- In both mice and rats, care must be taken to ensure adequate hemostasis following the procedure.
- Use of sterile capillary tubes and pipettes are recommended for use to help avoid periorbital infection and potential long-term damage to the eye. The edges of the tubes should be checked for smoothness to also decrease likeliness of eye damage.

Facial Vein Sampling (*limited to adult mice*): [9,24,25,26]

- Obtainable blood volumes: medium to large.
- Repeated sampling is possible by alternating sides of the face.
- Sample may be a mixture of venous and arterial blood.
- Manual restraint of awake animals results in proper site alignment and venous compression for good blood flow.
- 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.
- Clinical chemistry values may be higher with this method than with the retro-orbital plexus
- route.[14]

Saphenous Sampling (medial or lateral approach):[27,28,29,30]

- Can be used in both rats and mice by piercing the saphenous vein with a needle.
- Obtainable blood volumes: small to medium.
- Variable sample quality.
- The procedure is customarily done on an awake animal but 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:[31,32]

- Can be used in both rats and mice by cannulating the blood vessel or by superficially nicking the vessel perpendicular to the tail.
- 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 (cannulation) minimizes contamination of the sample, but is more difficult to perform in the mouse.
- 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.

- 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.
- Cannulation and tail nicking are routinely done without anesthesia, although effective restraint is required.

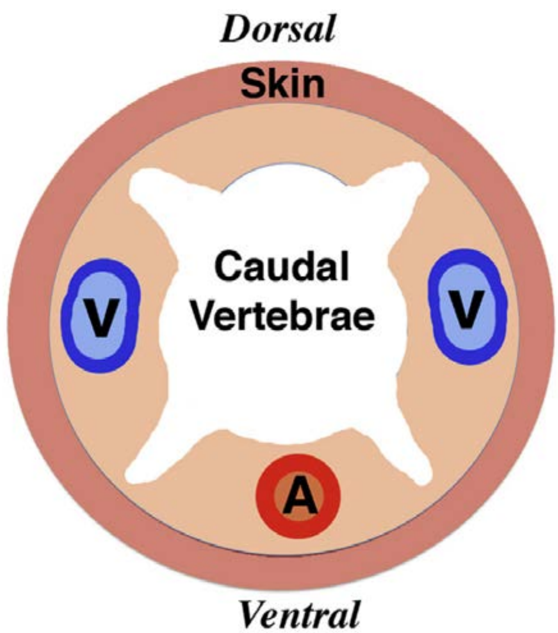

Figure 1. Cross-section of rodent tail, showing vessels used for blood collection.[6]

Tail Clip Sampling:[33]

- 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.
- Obtainable volume: small.
- 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.

Jugular Sampling (limited to the rat):

- Obtainable blood volumes: medium to large.
- Results in high quality sample.
- Jugular sampling can be conducted without anesthesia, although the use of anesthesia greatly facilitates the procedure.
- Does not easily lend itself to repeated serial sampling.

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Approved - 02/14/01
Revised - 01/12/05, 12/18/07, 09/03/08, 09/08/10, 09/12/12, 08/12/15

Table 2: Summary of Rodent Blood Sampling Techniques									
Route	**General Anesthesia Required**	**Speed & Efficiency**		**Sample Quality**		**Repeated Sampling**	**Relative Volumes Obtainable**	**Species**	**Comments**
		Mouse	**Rat**	**Mouse**	**Rat**				
Retro-orbital	Mouse - Recommended[1] Rat - Yes[2]	+++	++	+++	++	Should alternate eyes.	Medium to Large	Rat, Mouse	Rapid. Potential for complications.
Facial Vein	No	+++	N/A	+++	N/A	Yes	Medium to Large	Mouse	Rapid, easy, & repeated samples possible.
Saphenous Vein	No	++	++	++	++	Yes	Small to Medium	Rat, Mouse	Not as rapid as other techniques. Low potential for tissue damage.
Tail Vein or Artery	No	++ Vein +++ Artery	++ Vein +++ Artery	+ to ++[3]	++ to +++	Yes	Small to Medium (vein) Medium to Large (artery)	Rat, Mouse	Repeatable & simple. Variable sample quality.
Tail Clip	No	+++	+++	+/-	+/-	Yes	1-2 drops	Rat, Mouse	Repeatable if gently pull scab.
Jugular	Recommended	N/A	+/++	N/A	+++	Difficult	Large	Rat	Limited application. Poor for repeated sampling.

[1]Topical anesthesia must be used if general anesthesia is not used.

[2]Topical anesthesia is recommended in addition to general anesthesia.

[3]Depending upon method and amount of manipulation.