Guidelines for Survival Bleeding of Mice and Rats

These guidelines have been developed to assist investigators and institutional Animal Care and Use Committees (ACUC) in their choice and application of survival rodent bleeding techniques. The guidelines are based on peer-reviewed publications as well as on data and experience accumulated at NIH. It is the responsibility of both the investigator and ACUC to use techniques and procedures which result in the least pain and distress to the animal, while adequately addressing the needs of the experimental design. **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 ACUC should establish lines of accountability to oversee the training of its personnel. The procedures utilized must be reviewed and approved by the 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.
- The type of the sample required (eg. serum, whole cells, etc.).
- The quality of the sample required (sterility, tissue fluid contamination, etc.)
- The quantity of blood required.
- The frequency of sampling.
- Health status of the animal being bled.
- The training and experience of the phlebotomist.

The acceptable quantity and frequency of blood sampling is dependent on the circulating blood volume of the animal. 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, and 1% every 24 hours. Volumes greater than recommended should be justified in the ASP and appropriate fluid replacement provided. Blood sample ranges, based on body weight, are provided in Table 1 and recovery periods in Table 2.

Table 1: Approximate Blood Sample Volumes Ranges

Body weight (g)	*CBV(ml)	1% (ml)	10% (ml)
20	1.10 - 1.40	.011 - .014	.11 - .14
25	1.37 - 1.75	.014 - .018	.14 - .18
30	1.65 - 2.10	.017 - .021	.17 - .21
35	1.93 - 2.45	.019 - .025	.19 - .25
40	2.20 - 2.80	.022 - .028	.22 - .28
125	6.88 - 8.75	.069 - .088	.69 - .88
150	8.25 - 10.50	.082 - .105	.82 - 1.0
200	11.00 - 14.00	.11 - .14	1.1 - 1.4
250	13.75 - 17.50	.14 - .18	1.4 - 1.8
300	16.50 - 21.00	.17 - .21	1.7 - 2.1
350	19.25 - 24.50	.19 - .25	1.9 - 2.5

*Circulating blood volume

Table 2: Approximate Blood Sampling Volumes and Recovery Periods

Single Sampling		Multiple Sampling	
% Circulatory Blood Volume Removed	**Approximate Recovery Period**	**% Circulatory Blood Volume Removed In 24 hr**	**Approximate Recovery Period**
7.5%	1 Week	7.5%	1 Week
10%	2 Weeks	10 - 15%	2 Weeks
15%*	4 Weeks	20%*	4 Weeks

The following guidelines refer to the most frequently used survival sampling sites: a) tail; b) retroorbital; c) saphenous; d) jugular; and e) mandibular. Blood withdrawal by cardiac puncture is considered a euthanasia procedure and should be performed only after ensuring that the animal is under deep anesthesia. 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 3.

Lateral Tail Vein or Ventral/Dorsal Artery Sampling or Tail Clip:

- Can be used in both rats and mice by cannulating the blood vessel or by nicking it superficially perpendicular to the tail.
- Obtainable volumes (vein/artery): vein – small; artery – medium to large.
- Sample collection using a needle minimizes contamination of the sample, but is more difficult to perform in the mouse.
- 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 and skin products.
- Sample quality decreases with prolonged bleeding times and “milking” of the tail.
- Repeated collection possible
- Relatively non-traumatic
- Routinely done without anesthesia, although effective restraint is required.
- In most cases warming the tail with the aid of a heat lamp or warm compresses will increase obtainable blood volume.
- 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.
- For a very small sample, i.e., a single drop of blood, snipping of no more than the distal 1-*2* mm of the tail can be a viable alternative. With this method, the clot/scab can be gently pulled for repeat, small samples if needed for blood glucose measures, etc.

Retro-orbital Sinus/Plexus Sampling:

- Can be used in both rats and mice (though usually not a method of choice in the rat) by penetrating the retro-orbital plexus/sinus with a glass capillary tube or Pasteur pipette.
- Although the procedure may appear to members of the lay community as unduly distressful, the NIH ARAC has determined that in the hands of a skilled technician retro-orbital bleeding is a humane procedure that produces minimal and transient pain/distress.
- Rapid – large number of mice 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.
- Not amenable to frequent repeated sampling from the same orbit (10 days to 2 weeks recommended between successive bleeds).
- In the hands of an unskilled technician, retro-orbital sampling has a greater potential than other blood collection routes to result in complications.
- The presence of a plexus rather than sinus in the rat can lead to greater orbital tissue damage than in the mouse.
- Retro-orbital bleeding can be conducted in awake mice. A topical ophthalmic anesthetic should be applied prior to the procedure. Alternatively, systemic anesthesia should be considered if compatible with experimental design.
- Due to pain and distress issues retro-orbital sampling in the rat is best conducted under general anesthesia.
- In both mice and rats, care must be taken to ensure adequate hemostasis following the procedure.
- 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.

Saphenous Sampling (medial or lateral approach):

- Can be used in both rats and mice by piercing the saphenous vein with a needle.
- Obtainable blood volumes: small to medium.
- Repeated/serial sampling is possible
- Variable sample quality.
- The procedure is customarily done on an awake animal but effective restraint is required.
- Relatively low throughput technique compared to retro-orbital sampling due to time required for adequate site preparation (shaving).
- 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.
- Site clots/scabs can be gently pulled to provide small daily samples.

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 lend itself to repeated serial sampling.

Mandibular (Facial vein/artery) Sampling (*limited to adult mice*):

- 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.
- Requires less hands-on training than tail or retro-orbital sampling to reliably withdraw a reasonable quantity of blood.
- Perform on awake animals to achieve proper restraint which in turn 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 throughput.
- Sample volume can be partially controlled with the size of needle used to puncture the site.
- Excessive bleeding can be avoided by using a 20 gauge or smaller size needle.

References

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Table 3: Summary of Blood Sampling Techniques

Route	General anesthesia required	Speed and efficiency		Sample quality		Repeated sampling	Relative volumes obtainable	Potential for complications	Species	Comments
		Mouse	Rat	Mouse	Rat					
Tail Vein or Artery	No	++ Vein +++ Artery	+++ Vein +++ Artery	± to ++[1]	++ to +++	Yes	Small to medium (vein) Medium to large (artery)	Low	Rat, Mouse	Repeatable, simple, variable sample quality
Tail clip	No	+++	+++	+/-	+/-	Yes	1-2 drops	Low	Rat, Mouse	Repeatable if gently pull scab
Retro-orbital	Mouse – no[2] Rat - yes	+++	++	+++	++	Should alternate eyes	Medium to large	Moderate to high	Rat, Mouse	Rapid, potential for complications
Saphenous	No	++	++	++	++	Yes	Small to medium	Low	Rat, Mouse	Not as rapid as other techniques, low potential for tissue damage
Jugular	Recommended	N/A	+ /++	N/A	+++	Difficult	Large	Low	Rat	Limited application, poor for repeated sampling
Mandibular	No	+++	N/A	+++	N/A	Yes	Medium to large	Low	Mouse	Rapid, easy and repeated samples possible

[1] Depending on method and amount of manipulation

[2] Topical anesthesia recommended