

Guidelines for Survival Bleeding of Mice and Rats

Scope

- The purpose of these guidelines is to assist investigators in their choice and application of survival rodent bleeding techniques.
- Techniques and procedures which result in the least pain and distress to the animal while adequately addressing the needs of the experimental design must be used.
- The phlebotomist must be adequately trained.

General

Factors to consider in choosing the blood withdrawal technique include:

- Species
- Size of the animal
- Type of the sample required (e.g. serum, whole cells, etc.)
- Quality of the sample required (sterility, tissue fluid contamination, etc.)
- Quantity of blood required
- Frequency of sampling
- Health status of the animal
- Training and experience of the phlebotomist
- Effect of restraint or anesthesia on the blood parameter measured

Procedure

- 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 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 study protocol and appropriate fluid and/or cellular replacement provided.

Table 1: Approximate Blood Sample Volumes for a Range of Body Weights

Body weight (g)	*CBV(ml)	1% CBV (ml) every 24 hrs†	7.5% CBV (ml) every 7 days†	10% CBV (ml) every 2 - 4 wks†
20	1.10 - 1.40	0.011 - 0.014	0.082 - 0.105	0.11 - 0.14
25	1.37 - 1.75	0.014 - 0.018	0.10 - 0.13	0.14 - 0.18
30	1.65 - 2.10	0.017 - 0.021	0.12 - 0.16	0.17 - 0.21
35	1.93 - 2.45	0.019 - 0.025	0.14 - 0.18	0.19 - 0.25
40	2.20 - 2.80	0.022 - 0.028	0.16 - 0.21	0.22 - 0.28
125	6.88 - 8.75	0.069 - 0.088	0.52 - 0.66	0.69 - 0.88
150	8.25 - 10.50	0.082 - 0.105	0.62 - 0.79	0.82 - 1.0
200	11.00 - 14.00	0.11 - 0.14	0.82 - 1.05	1.1 - 1.4
250	13.75 - 17.50	0.14 - 0.18	1.0 - 1.3	1.4 - 1.8
300	16.50 - 21.00	0.17 - 0.21	1.2 - 1.6	1.7 - 2.1
350	19.25 - 24.50	0.19 - 0.25	1.4 - 1.8	1.9 - 2.5

*Circulating blood volume †maximum sample volume for that sampling frequency

Blood Draw Techniques

- The most frequently used survival sampling sites are retro-orbital, mandibular, saphenous, tail, and jugular.
- 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).
- 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.

Retro-orbital Sinus/Plexus

- Can be used in both mice and rats (though usually not a method of choice in the rat) by penetrating the retro-orbital sinus in mice or plexus in rats with a glass capillary tube or Pasteur pipette.
- Large numbers of mice can be bled within a short period of time.
- Medium to large volumes can be obtained.
- The quality of the sample is generally good. There is potential for contamination when topical anesthetic is used.
- Repeat sampling from the same orbit may be difficult (10 days to 2 weeks is recommended between successive bleeds). However, alternating orbits should not be attempted until the phlebotomist is proficient with the technique in the same orbit.
- In the hands of an unskilled phlebotomist, 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 if a topical ophthalmic anesthetic is applied prior to the procedure. Alternatively, systemic anesthesia should be considered if compatible with the 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 should be used 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 likelihood of eye damage.

Mandibular (Facial vein/artery, limited to adult mice)

- Medium to large volumes can be obtained.
- Repeated sampling is possible by alternating the sides of the face.
- Samples 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.
- Can be performed on awake animals.
- Proper restraint 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 volumes can be partially controlled with the size of instrument used to puncture the site (needle, 20 gauge or smaller, or lancet, 4 mm).

Saphenous (medial or lateral approach)

- Can be used in both rats and mice by piercing the saphenous vein with a needle.
- Small to medium volumes are usually obtained.
- Variable sample quality.
- The procedure is usually done on an awake animal but effective restraint is required.
- Longer time to perform 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.
- The clot/scab can be gently removed for repeated small samples if serial collection is required.

Lateral Tail Vein or Ventral/Dorsal Artery or Tail Clip

- Can be used in both rats and mice by cannulating the blood vessel, by superficially nicking the vessel perpendicular to the tail or by clipping (i.e., amputating) 1-2 mm of the distal tail.
- Sample collection by nicking the vessel or clipping the distal tail 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 collections are possible. With tail nicking/clipping, the clot/scab can be gently removed for repeated small samples if serial testing is required (e.g., glucose measurements, etc.).
- 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.
- Obtainable volumes for tail clipping are small.
- Relatively non-traumatic.
- In most cases, warming the tail with the aid of a heat lamp or warm compresses will increase obtainable blood volume.
- Cannulation and tail nicking are routinely done without anesthesia, although effective restraint is required.
- 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 (limited to the rat)

- Medium to large volumes can be obtained.
- Results in high quality samples.
- 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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