Guidelines for survival bleeding of mice and rats

These Guidelines have been developed to assist investigators and institutional Animal Care and Use Committees (IACUC) 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
It is the responsibility of both the investigator and IACUC 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 IACUC should establish lines of accountability to oversee the training of its personnel. The procedures utilized must be reviewed and approved by the IACUC 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 (e.g. 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.

Both the quantity and frequency of blood sampling is dependent on the circulating blood volume of the animal.
In general, no more than 10% of the animal's blood volume should be removed at one sampling. Volumes greater than 10% should be justified in the experimental protocol and appropriate fluid replacement considered. Suggested recovery periods vs. blood sample size are provided in Table I.

The following guidelines refer to the most frequently used survival sampling sites:

a) Tail
b) Retro-orbital
c) Saphenous
d) Jugular.
e) Facial Vein

Blood withdrawal by cardiac puncture is considered a terminal procedure and should be performed only after ensuring that the animal is under deep anesthesia.

Retro-orbital bleeding in mice

This procedure will be done only IN THE HANDS OF A SKILLED PRACTITIONER. Retro-orbital bleeding is a humane method to obtain blood samples from mice. Investigators should be aware that this procedure may cause transient pain and distress and appears to members of the lay community and others to be unpleasant to the animal. Training by the attending veterinarian, or his/her designee, is absolutely required to achieve proficiency in retro-orbital bleeding. The attending veterinarian will grant authorization for an investigator to perform retro-orbital bleeding only after an individual has become proficient at the procedure.
Alternative methods* of blood collection such as saphenous or tail vein puncture are available, and investigators are encouraged to consider these as alternatives to retro-orbital bleeding.

All blood sampling (including frequency of sampling and volume of sample) must be approved in the IACUC application.

Ideally, mice should be at least three weeks of age.

Procedure

1) Manually restrain mouse by grasping near base of tail with one hand and grasping the nape of the neck with the opposite hand. Place tail between fingers to secure and control animal.
2) Apply one drop of topical ophthalmic anesthetic (e.g., proparacaine, tetracaine) to eye to be used. Wait a few seconds. (An alternative to topical anesthesia is general anesthesia.)

3) Place hematocrit tube or Pasteur pipette at the canthus of eye. Sterile hematocrit tubes or Pasteur pipettes are recommended for use, but are required for immune compromised animals. It is imperative that you know the volume of the tube you are using to collect blood and that you do not exceed the approved volume for the weight of the animal. For example, the volume of a standard microhematocrit tube is 70 microliter and the volume of a Pasteur pipette to the tapered section (shoulders) is 250 microliter.
4) With a gentle rotating motion, insert tube through membrane.

5) Continue rotating tube on back of orbit until blood flows.

6) In most cases, no measures need to be taken to ensure good hemostasis. Should excessive bleeding occur, apply gentle pressure with a gauze pad, being careful to avoid scratching the cornea. No more than 10% of the blood volume should be removed at one sampling.

7) Blood volume of a mouse is ~8% of the body weight. For example a 25 gm mouse has a blood volume of ~2 ml - not more than 200 µl could be removed at a single bleeding without scientific justification and approval by IACUC.
Mice should not be bled any more frequently than every 3 weeks unless smaller volumes are removed.

Facial vein bleed in mice

1. Equipment

a. Collection of blood is from a free catch into a blood tube or an Eppendorf tube. In some cases, it is desirable to collect blood directly into hematocrit tubes.

b. Goldenrod lancets will be selected based on the appropriate size for the animal according to age and sex.

1. Lancets are selected according to the age/size of mice as follows:
   4mm lancet: 3-4-week-old mice (under 15 grams body weight)
   5mm lancet: female mice under 10 weeks (under ~20 grams body weight)
   5mm lancet: male mice under 6 weeks (under ~20 grams body weight)
   5mm lancet: for single-drop samples (for blood smears)
   5.5mm lancet: female mice over 10 weeks (over ~20 grams body weight)
   5.5mm lancet: male mice over 6 weeks (over ~20 grams body weight)
   5.5mm lancet: large samples

2. Restraint

a. Mice are restrained using the scruffing technique.

b. It is important that side-to-side movement of the head be minimized. This ensures accurate and safe venipuncture with the lancet.

3. Blood withdrawal

a. The proper-size lancet is held perpendicular to the surface of the skin.

b. The lancet point is slightly angled, with the tip facing towards the nose.

c. The lancet blade is best used in a vertical position.

d. While restraining the mouse, locate the approximate area of the facial vein by measuring the length of the eye below the lateral canthus and the width of the eye caudally.

e. With the point of the lancet, gently feel for the point at which the jawbone ends.

f. For better accuracy in puncturing the vessel, position the mouse in lateral recumbence.

g. Pierce the skin to the shoulder of the lancet at that point. This is done with a firm push and not as throwing a dart.

h. Upon removal of the lancet, blood will begin to flow.

i. To assist blood flow, position the mouse with the head lower than the heart.

j. Collect the blood in the desired collection vessel.

k. Blot the puncture site and release pressure on the scruff for hemostasis.

Lateral tail vein, ventral/dorsal artery sampling:

Can be used in both rats and mice by cannulating the blood vessel or by nicking it superficially perpendicular to the tail.
Obtainable volume: Mouse - small to medium
Rat - medium
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 a one-time collection of a very small sample, i.e., a single drop of blood, snipping of no more than the distal 1 mm of the tail can be a viable alternative.

Saphenous/lateral tarsal sampling:

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. Prolonged restraint and site preparation time can result in increased animal distress when handling an awake animal.
Temporary favoring of the limb may be noted following the procedure.

Jugular sampling:

Limited to the rat.
Obtainable blood volumes: medium
to large. High sample quality.
Jugular sampling can be conducted without anesthesia, although the use of anesthesia greatly facilitates the procedure.
Does not lend itself to repeated serial sampling.

Table I: Blood Sampling Volumes and Recovery Periods

Single Sampling	Single Sampling	Multiple Sampling	Multiple Sampling
% Circulatory Blood Volume Removed	**Approximate Recovery Period**	**% Circulator y 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

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