Guidelines for Rodent Blood Collection

These guidelines have been developed to assist investigators and the NCI at Frederick Animal Care and Use Committee (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 the NCI at Frederick. It is the responsibility of the investigator to use techniques and procedures that result in the least pain and distress to the animal, while adequately addressing the needs of the experimental design. Training and experience of the technician in the chosen procedure are of paramount importance. Training opportunities and resources, including access to experienced personnel, are available to new personnel. Blood collection must be recorded on the cage card. The procedures utilized must be reviewed and approved by the ACUC prior to their implementation.

Factors to consider in choosing the blood withdrawal technique include:

- 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 technician

<u>Permissible Collection Volumes</u>: Both the quantity and frequency of blood sampling is dependent on the circulating blood volume of the animal. The approximate blood volume of a mouse is 80 ml/kg and 70 ml/kg for the rat. Please see the tables (1 and 2) below for permissible blood volume removal amounts.

<u>For a single survival collection</u>: If you require more than 13% of an animal's blood volume, a scientific justification is required in advance for ACUC review and approval.

<u>For a single collection followed by immediate euthanasia:</u> If you require more than 35% of an animal's blood volume [this is based on a human calculation for allowable blood loss for which a range of 20 – 43% is acceptable blood loss depending on the starting hematocrit], the animal must be anesthetized for the procedure followed by humane euthanasia. If anesthesia cannot be used, a scientific justification is required in advance for ACUC review and approval.

<u>For multiple survival collections</u>: If you require more than 13% of an animals' blood volume in any given two-week timeframe, a scientific justification is required in advance for ACUC review and approval.

Please use the chart below or the following calculation to determine the permissible sample volume (13%) that can be collected for your study: volume [ml] = 0.01 [mice] or 0.0091 [rats] x Animal's Body Weight [in gm]

TABLE 1 - MOUSE	
Body Weight at the time of collection	**Permissible Sample Volume 13%**
20g	200 ul
30g	300 ul
40g	400 ul
Frequency	Every 2 weeks

TABLE 2 - RAT	
Body Weight at the time of collection	**Permissible Sample Volume 13%**
100g	0.91 ml
200g	1.82 ml
300g	2.73 ml
Frequency	Every 2 weeks

NOTE: Volumes exceeding amounts listed above will require justification to the ACUC and may require fluid replacement in the form of an equal volume of warm saline (SQ) will be administered to the animal

The following guidelines refer to the most frequently used survival sampling sites: a) Tail; b) Mandibular c c) Saphenous and; d) Retro-orbital. Blood withdrawal by cardiac puncture or axillary cut down are considered terminal procedures and must be performed only after ensuring that the animal is under surgical anesthesia. Issues that should guide the choice of survival blood collection route(s) are listed below, and an abbreviated summary is provided as Table 3. It is important to note that samples collected from different sites may show differences in clinical pathology values. Please refer to the information above regarding permissible collection volumes.

A. Lateral Tail Vein or Ventral/Dorsal Artery:

- 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 [50-100 ul]
  Rat – medium [0.2-0.4 ml]
- 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 tail stroking.
- 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.
- Arterial sampling produces larger volumes and is faster, but special care must be taken to ensure adequate hemostasis.
- Piercing the tail vein with a needle is also a way to collect a very small blood sample.

B. Mandibular Vein/Artery:

- Can be used in both rats and mice by piercing the mandibular vein or artery with a needle [20G] or stylet.
- Obtainable volume: medium to large [100-200 ul, mouse; 0.4-0.5 ml rat]
- Repeat sampling is possible.
- Sample quality is good.
- The procedure is customarily done on an unanesthetized animal, but effective restraint is required.
- Arterial sampling produces large volumes very rapidly.
- Venous sampling produces medium volumes more slowly.
- Ensure that gentle pressure is applied for approximately 30 seconds post-collection to ensure hemostasis.

C. Saphenous/Lateral Tarsal:

- Can be used in both rats and mice by piercing the saphenous vein with a needle [23-25G: mouse, 21-23G: rat].
- Obtainable blood volumes: small to medium [mouse: 100 ul; rat: 0.4 ml]
- Repeat sampling is possible.
- Variable sample quality.
- The procedure is customarily done on an unanesthetized animal, but effective restraint is required.
- Can be more time-consuming than other methods due to time required for site preparation.
- After training, it requires more practice 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 unanesthetized animal.
- Temporary favoring of the limb may be noted following the procedure.
- Care must be taken to ensure adequate hemostasis following the procedure.

D. Retro-orbital:

*Note: Due to the increased risk of complications associated with this procedure, the ACUC recommends that other routes of blood collection be considered prior to use of this method. The mandibular technique permits an equivalent volume of blood to be collected in a rapid manner with less risk or complications.

- Individuals performing the procedure must be certified by LAM.
- Can be used in mice by penetrating the retro-orbital sinus with a glass capillary tube [0.5 mm in diameter] or via the retro-orbital plexus in rats with a capillary tube.
- Must be performed by a skilled operator.
- Follow-up required 24-48 hours after blood collection. If complications such as squinting or bulging of the eye are noted, an animal health report must be completed.
- Obtainable volume: medium to large
- Collection is limited to once per eye.
- In the hands of an unskilled operator, retro-orbital sampling has a greater potential than other blood collection routes to result in the following complications:

  - Hematoma and excessive pressure on the eye resulting from retro-orbital hemorrhage
  - Corneal ulceration, keratitis, rupture of the eyeball or micro-ophthalmia caused by pressing on the eye to stem persistent bleeding or from a hematoma
  - Damage to the optic nerve and other intra-orbital structures leading to vision deficits or blindness
  - Fracture of the bones of the orbit and neural damage by the pipette; loss of vitreous humour due to penetration of the eyeball

- Skilled personnel can conduct retro-orbital bleeding in unanesthetized mice. Anesthesia is recommended for retro-orbital blood collection in mice and is required during the training of personnel.
- It is highly recommended to use a topical ophthalmic ointment, such as proparacaine or tetracaine drops, 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 ACUC. In addition, a topical ophthalmic anesthetic, e.g. proparacaine or tetracaine, is recommended prior to the procedure. Retro-orbital bleeding performed in rats by a trained practitioner represents more than "minimal or transient pain or distress" and therefore should be considered a Category 2 procedure.
- Care must be taken to ensure adequate hemostasis following the procedure.

Terminal and post-mortem blood collection

Blood withdrawal by cardiac puncture or axillary cut down are considered terminal procedures and must be performed only after ensuring that the animal is under surgical anesthesia. The post-mortem collection from the aorta is performed immediately after euthanasia.

A. Cardiac Puncture

- Can be used in both rats and mice by penetrating the heart.
- Must be performed by a skilled operator.
- Obtainable volume: medium to large.
- Animal must be euthanized immediately after blood collection.

B. Axillary cut down

- Can be used in both rats and mice.
- Axillary vessels are cut with a scalpel blade **or scissors** and the pooled blood is collected via capillary tube.
- Obtainable volume: medium to large.
- Animal must be euthanized immediately after blood collection prior to recovery from anesthesia.

C. Pre-mortem collection from the aorta or vena cava

- Can be used in both rats and mice as a pre-mortem procedure on anesthetized animals.
- Blood is collected using a needle.
- Animal must be euthanized immediately after blood collection prior to recovery from anesthesia.
- Obtainable volume: medium to large.

D. Post-mortem collection from the aorta

- Can be used in both rats and mice as a post-mortem procedure in a euthanized animal.
- Must be done rapidly after euthanasia to ensure blood flow.
- Aorta is cut and the blood pools in the pleural cavity.
- Blood is collected in a mini capillary tube. The tube must be held continuously in a horizontal position during the blood draw.
- Obtainable volume: medium to large.

Table 3: Summary of Blood Sampling Techniques

Route	Anesthesia Required		Speed		Sample Quality		Repeat Samples		Relative Obtainable Volume *(approximations)*		Potential for Complications	
	Mouse	Rat	Mouse	Rat	Mouse	Rat	Mouse	Rat	Mouse	Rat	Mouse	Rat
Tail Vein	No	No	Med	Med	Fair	Good	Yes	Yes	Small (50 ul)	Small *(.2 mls)*	Low	Low
Tail Artery	No	No	Fast	Fast	Good	Very Good	Yes	Very Good	Medium *(100 ul)*	Medium *(.4 mls)*	Low	Low
Retro-orbital	No	Yes	Fast	Med	Very Good	Good	Alternate eyes	Alternate eyes	Med.-Large *(200 ul)*	Med.-Large *(.5 mls)*	Moderate -High	Moderate -High
Saphenous	No	No	Med.	Med	Good	Good	Yes	Yes	Small-Med. *(100 ul)*	Small-Med. *(.4 mls)*	Low	Low
Mandibular Vein	No	No	Slow-Med.	Slow-Med.	Fair-Good	Fair-Good	Yes	Yes	Small-Med. *(100 ul)*	Small-Med. *(.4 mls)*	Moderate	Moderate
Mandibular Artery	No	No	Very Fast	Very Fast	Very Good	Very Good	Yes	Yes	Large *(200 ul)*	Large *(.5 mls)*	Moderate	Moderate

REFERENCES

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