These guidelines have been developed to assist investigators and the Howard University Institutional Animal Care and Use Committee (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 accumulated at the Frederick National Lab. 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 IACUC 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

Permissible Collection Volumes

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.

For a single survival collection: If you require more than 13% of an animal's blood volume, a scientific justification is required in advance of the performing the procedure. Please submit to the IACUC review and approval.

For a single collection followed by immediate euthanasia: 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 IACUC review and approval.

For multiple survival collections: 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 OF THE TIME OF COLLECTION	PERMISSIBLE SAMPLE VOLUME 13%
20g	200ul
30g	300ul
40g	400ul
Frequency	Every 2 weeks

TABLE 2-RAT	
BODY WEIGHT OF THE TIME OF COLLECTION	**PERMISSIBLE SAMPLE VOLUME 13%**
100g	0.91 ml
200g	1.82 ml
300g	2.72 ml
Frequency	Every 2 weeks

NOTE: Volumes exceeding amounts listed in the above two tables will require justification to the IACUC 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. 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.

B. 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.

C. Lateral Tail Vein or Ventral/Dorsal Artery:

- Can be used in both rats and mice by inserting a 23G needle or by nicking it superficially perpendicular to the tail respectively.
- This technique requires that the animal be warmed in order to dilate the blood vessel prior to taking the sample. This may be stressful and can cause dehydration due to salivation, in addition to increasing metabolic rate, which may affect the experimental data.

- Aseptic technique should be used. It is recommended to apply a local anesthetic cream (e.g. EMLA cream) to the site 30 minutes prior to blood sampling.
- 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. It is not recommended rat for repeat samples
- 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 the rat arterial collection requires analgesia and anesthesia, however it yields larger volumes and is faster, but special care must be taken to ensure adequate hemostasis. A cannula is recommended for repeat samples.
- If the animal was anesthetized, monitor the animal until it is fully awake and able to walk normally.
- Clean restraint devices between animals.

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 VS.
- 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

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
- 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.

REFERENCES

1. Perspectives on Animal Use Biological Effects of Blood Loss: Implications for Sampling Volumes and Techniques. In: ILAR News (1989), 31(4).
2. Removal of blood from laboratory mammals and birds: First report of the BVA/FRAME/RSPCA/UFAW Joint working group on refinement. Lab Animal (1993) 27, 1-22.
3. J. Donovan and P. Brown. Blood Collection. In: Current Protocols in Immunology, (eds: A. Kruisbeek et al), John Wiley & Sons, New York, NY, USA, Unit 1.7.
4. H. van Herck et al., Orbital sinus blood sampling in rats as performed by different technicians: the influence of technique and expertise. Lab Animal (1998) 32, 377-386.
5. http://www.eslav.org/efpia.htm
6. "Guidelines for the Survival Bleeding of Mice and Rats." ARAC Guidelines (Revised September 2010).
7. http://ncifrederick.cancer.gov/Lasp/acuc/Frederick/Media/Documents/ACUC20.pdf
8. www.nc3rs.org.**uk/blood**samplingmicrosite/page.asp?id=1335