

## **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.

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 72 ml/kg  $\pm$  8 ml and 64 ml/kg  $\pm$  6 ml for the rat (e.g., 1.5 ml for a 20 gr mouse and 13 ml for a 200 gr rat). 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 ASP 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 and d) Jugular. Blood withdrawal by cardiac puncture is considered a terminal procedure and should be performed only after ensuring that the animal is under deep anesthesia. Issues that should guide the choice of survival blood collection route(s) is listed below, and an abbreviated summary is provided as Table II.

Lateral Tail Vein or 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.

Retro-orbital 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.
- The NIH ARAC has determined that in the hands of a skilled operator 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 operator, 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 restraint issues retro-orbital sampling in the rat should be conducted under general anesthesia.
- In both mice and rats, care must be taken to ensure adequate hemostasis following the procedure.

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<sup>\*</sup>

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

\*With higher withdrawal volumes, additional monitoring (e.g. hematocrit, hemoglobin) and appropriate fluid replacement should be considered.

Table II: 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 ++ <sup>1</sup>	++ to +++	yes	small (vein) medium (artery)	low	Rat, Mouse	Repeatable, simple, variable sample quality
Retro-orbital	Mouse – no <sup>2</sup> Rat- yes	+++	++	+++	++	difficult	medium to large	moderate to high	Rat, Mouse	Rapid, potential for complications
Saphenous/ Lateral Tarsal	no	++	++	++	++	yes	small to medium	low	Rat, Mouse	Not as rapid as other techniques, low potential for tissue damage
Jugular	Recom- mended		+ /++		+++	difficult	large	low	Rat	Limited application, poor for repeated sampling

<sup>1</sup> Depending on method and amount of manipulation

<sup>2</sup> Topical anesthesia recommended

## 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 Anim (1993) 27, 1-22.
3. J. Donovan and P. Brown. Blood Collection. In: Current Protocols in Immunology, (eds: A. Krusbeek 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 Anim (1998) 32, 377-386.
5. <http://www.eslav.org/efpia.htm>
6. [http://www.uib.no/vivariet/mou\\_blood/Blood\\_coll\\_mice\\_.html](http://www.uib.no/vivariet/mou_blood/Blood_coll_mice_.html)