



**THE UNIVERSITY OF TOLEDO
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE**

SUBJECT: Blood Collection Guideline

DATE: April 21, 2021

Blood Collection Guideline

This guideline establishes safe blood collection practices for laboratory animals. Blood collection procedures must be described in the approved Institutional Animal Care and Use Committee (IACUC) protocol, including the method, the volume of blood to be collected, and the interval between blood collection events. The method and volume of blood to be collected will depend on the animal species, frequency of collection, and experimental needs. Researchers should take measures to minimize stress associated with the blood collection procedure and blood loss on the physiology of the animal. This will also help to minimize experimental variables and confounding influences on research data. Any exceptions to these guidelines, such as increase in blood volume or frequency to be collected, must be scientifically justified in the protocol. (1,2) Researchers should contact DLAR for blood collection procedure training.

Table 1. Approximate Circulating Blood Volume

<i>Species</i>	<i>Approximate blood volume</i>
Mouse	63 to 80 ml/kg body weight (mean 72 ml/kg)
Rat	58 to 70 ml/kg body weight (mean 64 ml/kg)
Rabbit	44–70 ml/kg body weight (mean 56 ml/kg)

Percentage of total circulating blood volume that can be safely removed and frequency is summarized in Table 2. “Frequency” represents the recovery period the animal needs in order to replenish cells lost during the bleed and to recover from the physiologic response to hemorrhage. If additional blood samples are needed, the animal may not be bled again until after the period of time stated in “Frequency”. Samples may be taken during a single bleeding event or during multiple events over time. If taken during multiple events (for example during a pharmacokinetics study over 96 hours or a glucose tolerance test over 2 hours), the total volume taken (all samples combined) cannot exceed 15%, and the “recovery period” starts after the last blood collection event. These recommendations do not apply to a terminal sample collection, where an animal is exsanguinated while deeply anesthetized and euthanized while still under anesthesia (must be approved in the IACUC protocol). (3)

Table 2. Example Volumes (using the mean blood volume)

Species	Example body weight	Total blood volume (ml)	7.5% (ml)	10% (ml)	15% (ml)
Mouse	25 g	1.8	0.135	0.180	0.270
Rat	250 g	16	1.2	1.6	2.4
Rabbit	4 kg	224	17	22	34
Frequency/recovery period			Every 7 days	Every 2 weeks	Every 4 weeks
Replacement fluids*			Recommended	Required	Required

Table 3. Blood Collection Techniques for Mice

Location	Volume that can be collected	Notes
Saphenous vein	Small to moderate	
Tail vein nick	Small to moderate	
Submental	Moderate to large	
Facial/submandibular	Moderate to large	
Cardiac puncture	Large	Anesthesia required, terminal collection only
Retro-orbital	**	**

Table 4. Blood Collection Techniques for Rats

Location	Volume that can be collected	Notes
Saphenous vein	Small to moderate	
Tail vein nick	Small to moderate	
Cardiac puncture	Large	Anesthesia required, terminal collection only
Retro-orbital	**	**

Table 5. Blood Collection Techniques for Rabbits

Location	Volume that can be collected	Notes
Cephalic	Moderate to large	
Marginal ear vein or central ear artery	Moderate to large	Local anesthesia recommended; sedation may be helpful
Cardiac puncture	Large	Anesthesia required, terminal collection only

* Warm Lactated Ringer's Solution (LRS) or 0.9% sterile isotonic saline

**** Retro-orbital blood collection**

Retro-orbital blood collection in rodents can provide moderate to large amounts of blood when performed by well-trained personnel. However, severe injuries may occur to the animal if this procedure is not done properly. Alternative collection sites are strongly recommended. Use of this collection method will need to be scientifically justified in the protocol.

If retro-orbital collection is necessary, the following guidelines apply:

- General anesthesia is required.
- Application of a topical ophthalmic anesthetic during/after collection should be considered to provide post-procedural analgesia.
- Microhematocrit tubes that hold 50 - 75 microliters are recommended to minimize risk of injury.

- If attempted collection from one eye is unsuccessful, an alternate method approved in the protocol (e.g. submandibular or saphenous route) must be used, rather than reattempting retro-orbital collection from the same or opposite eye.
- Alternate between left and right eyes per session.
- No more than 1 collection can be performed every 7 days (alternate eyes), thus, 14 days between collections in the same eye.
- A maximum of 3 procedures may be performed per eye (up to 6 collections total)
- If injury and/or rupture of the eye occurs, the animal must be immediately euthanized.
- Daily monitoring is required for 3 days post-procedure. (4)

References

1. <https://www.research.uky.edu/division-laboratory-animal-resources/guidelines-blood-collection-laboratory-animals>
2. McGill M.W. and Rowan A.N. Biological Effects of Blood Loss: Implications for Sampling Volumes and Techniques. ILAR News (1989), 31(4): 5-18
3. Diehl, K. H. et al. A good practice guide to the administration of substances and removal of blood, including routes and volumes. Journal of applied toxicology : JAT21, 15-23 (2001).
4. <https://animal.research.uiowa.edu/iacuc-guidelines-blood-collection>