



Title: Acceptable Methods of Blood Collection

Policy Number: 007

Policy Intent: 1) To assist investigators and the IACUC members in their choice and application of survival and non-survival rodent bleeding techniques during protocol formulation and review. 2) To describe the recommended blood collection volume and frequency from commonly used in laboratory animal species. This Policy is approved by the TTU IACUC and any deviation must be approved by the IACUC prior to its implementation.

Table of Contents

- 1. Introduction**
- 2. Procedures**
- 3. Guidelines for rodents**
- 4. Approved Methods**
- 5. Non-Approved Methods**
- 6. Appendix- Blood volumes for Various Species**
- 7. References**

1. Introduction

- A. This policy provides information about the volume and frequency of blood collection in various species. These techniques and maximum limits are set to maintain animal well-being and reduce the potential for complications such as hypovolemic shock and death.
- B. All methods of blood withdrawal should be previously approved by the IACUC in the form of an approved protocol.

2. Procedures

- A. The guidelines provided are for healthy, normal adult animals. The listed amounts of blood loss may be poorly tolerated by younger, aged, stressed or diseased animals.
- B. In all cases, non-terminal blood collection without fluid replacement is limited to 10% of the total circulating blood volume of a healthy animal during a 2 week period. If larger amounts are needed, then up to 15% of the total circulating blood volume may be withdrawn if replacement fluids are given at the time of blood withdrawal. Removal of 15% of total blood volume must be justified in the animal protocol and approved by the TTU IACUC.
- C. For large animals, removal of greater than 10% of total blood volume at a single withdrawal is not appropriate.
- D. Do not collect blood from a site that has evidence of inflammation.
- E. Use a minimal number of venipunctures per day with no more than two punctures per site unless otherwise justified in the protocol.
- F. All personnel performing these methods should be adequately trained prior to performing the technique. An Animal Care Services staff member or veterinarian should be consulted if training or assistance is required.



3. Guidelines for rodents

Table 1: Approximate Blood Sample Volumes for a Range of Body Weights.

**Please note, a veterinarian should be consulted for questions regarding blood sample volumes for rodents greater than 350g (i.e.- large adult rats)*

Body weight (g)	*CBV(ml) Circulating Blood Volume	1% CBV (ml) every 24 hrs†	7.5% CBV (ml) every 7 days†	10% CBV (ml) every 2 wks†
20	1.10 – 1.40	.011 – .014	.082 – .105	.11 – .14
25	1.37 – 1.75	.014 – .018	.10 – .13	.14 – .18
30	1.65 – 2.10	.017 – .021	.12 – .16	.17 – .21
35	1.93 – 2.45	.019 – .025	.14 – .18	.19 – .25
40	2.20 – 2.80	.022 – .028	.16 – .21	.22 – .28
125	6.88 – 8.75	.069 – .088	.52 – .66	.69 – .88
150	8.25 – 10.50	.082 – .105	.62 – .79	.82 – 1.0
200	11.00 – 14.00	.11 – .14	.82 – 1.05	1.1 – 1.4
250	13.75 – 17.50	.14 – .18	1.0 – 1.3	1.4 – 1.8
300	16.50 – 21.00	.17 – .21	1.2 – 1.6	1.7 – 2.1
350	19.25 – 24.50	.19 – .25	1.4 – 1.8	1.9 – 2.5
*Circulating blood volume		†maximum sample volume for that sampling frequency		

4. Acceptable Methods

Route	Species	General Anesthesia Required	Extra training required	Comments
Saphenous Vein Puncture	Rats, Mice, Dogs, Cats	No	No	Good for repeated small bleeds or one large bleed. Low potential for tissue damage.
Tail Vein Puncture	Rats, Mice, Cattle	No	No	Use of a needle preferred resulting in less tissue damage to tail
Cardiac Puncture	Rats, Mice	Yes	No	Non-Survival only; Animal must be deeply anesthetized and not recover from anesthesia
Cephalic Vein	Dogs, Cats, Pigs	No	No	Good for catheter placement
Facial Vein Puncture/Submandibular	Mice	No	Yes	Good for single large samples but some complications possible
Jugular Vein	Rats, Rabbits, Dogs, Cats, Farm Animals, Deer	Yes (rodents) No (others)	No	Limited application in rodents; Large samples in farm animals
Tail Clip (Biopsy)	Mice, Rats	Yes (after weaning)	No	Good for blood collection while genotyping. Up to 3mm of tail tip may be collected. Anesthesia may be required based on the



Institutional Animal Care and Use Committee™

				age of the animal (refer to IACUC Policy 17 for further explanation). No more than one tail clip is performed on a single animal.
Route	Species	General Anesthesia Required	Extra training required	Comments
Ear Vein	Rabbits, Pigs	No	Yes	Small, but repeated sampling; vein easily blown
Retro-orbital (Provisionally acceptable)	Mice	Yes	Yes	<p>Adverse Effects Eye infection, Peri-orbital swelling, Redness and/or hematoma formation, Corneal ulceration, keratitis, pannus formation, rupture of the globe and microphthalmia caused by proptosis of the globe, Damage to the optic nerve, Penetration of the eye globe, and Blindness</p> <p>Cautionary statements Retro-orbital sampling has a greater potential than other blood collection routes to result in complications A minimum of 10 days should be allowed for tissue repair (USDA Category D)</p>
Tail Snip	Mice only	No	Yes	<p>Good for small, multiple samples over a short period of time, by removing the clot/scab after the initial cut.</p> <p>Only soft tissue may be removed and no ossified vertebral tissue (up to 2 mm of fleshy tail tip only). Minimum of two weeks required in between tail snips to allow the animal to recover. No more than two tail snips are performed on a single animal.</p>

5. Non-Acceptable Methods

Route	Reasons for non-approval
Penile or Lingual Vein	Potential for thrombosis leading to blockage of the urethra or swelling of the tongue
Amputation of a body part: tail or toe (exception is single use of tail; see above)	Can lead to granuloma formation resulting in a mass at the end of the tail leading to pain; repeated amputation results in cutting of bone and samples that are hemolyzed and contaminated with other tissue.



Cardiac puncture as a survival procedure	Damage to lungs and/or heart, potential to lacerate major vessel resulting in death, bleeding into the pericardium resulting in cardiac arrest
--	--

6. Appendix- Blood Volumes for Various Species. Adapted from Wolfensohn & Lloyd, 2003, Handbook of Laboratory Animal Management and Welfare, 3rd Edition

Species	Reference Weight (g)	Blood volume (ml/kg) ^a	Circulating Blood Volume (CBV), normal adult (ml); 10% CBV q 2-4 weeks
Mouse	18-40	58.5	Male 1.5-2.4 ; (0.15-0.24 ml) Female 1.0-2.4; (0.1-0.24 ml)
Rat	250-500	54-70	Male 29-33; (2.9-3.3 ml) Female 16-19; (1.6-1.9 ml)
Ferret	750-1500	75	50-110; (5-11 ml)
Rabbit	1000-6000	57-65	160-480; (16-48 ml)
Chicken	1000-3000	60	60-180; (6-18 ml)
Quail	110-200	65-100	7.56-20; (0.75-2ml)
Dog		70-110 ^b	900-1170 ^c ; (90-117 ml)
Cat		47-65	140-200; (14-20 ml)
Pig		56-69	13,200-15,000 (1.3-1.5 L)
Sheep		58-64	4060-4480; (406-448 ml)
Goat		57-90	3990-6300; (390-630 ml)
Horse		75	33,750-45,000 ^d ; (33-45 L)
Cattle		60	27,000-36,000 ^d ; (27-36 L)
Deer	45,000-125,000	66 ^a	2970-8250; (297-825 ml)

^aBlood volume estimate for a single species may not reflect differences among individual breeds or variations due to age, size or illness

^bMuch breed variation

^cBeagle

^dAssumes adult weight 450-600 kg

7. References

- Wolfensohn, S., Lloyd, M. 2nd Edition, Blackwell Science Ltd. 1998.
- Guidelines for survival bleeding of mice and rats;
http://oacu.od.nih.gov/ARAC/documents/Rodent_Bleeding.pdf
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3189672/pdf/jaalas2011000680>.
- Cornell University IACUC ACUP 403 Recommended Blood Collection Volume and Frequency. <http://www.research.cornell.edu/care/documents/ACUPs/ACUP403.pdf>