University Animal Care Committee Standard Operating Procedure		
Document No: 7.10.1	**Subject: Saphenous Blood Collection in Mice**	
Date Issued: March 14, 2012	**Revision:** 6	**Page No:** 1

Location: Queen's University

Responsibility: Principal Investigators, Research Staff, Veterinary Staff

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe the methods of saphenous blood collection in mice.

1. Introduction and Definitions:

Abbreviations: Animal Care Services **ACS**, Principal Investigator **PI,** subcutaneous **SC**, intravenous **IV**, intraperitoneal **IP,** intramuscular **IM**, per os **PO,** per rectum **PR**

Use the following table to determine the most appropriate site for blood collection based on the volume required.

Site	Submandibular	Saphenous	Submental	Tail Vein	Retro-orbital	Cardiac Puncture
Multiple sampling	Yes	Yes	Yes	Yes	Yes	No
Volume	Max.200µl	Max. 200µl	Max. 200µl	50µl	Max. 200µl	TBV
Gauge Needle	4-5.5 mm lancet	23-25g	4-5.5 mm lancet	23-25g/scalpel	Capillary tube	23-25g

The following are "good practice" guidelines recommended for blood collection volumes, sites and needle gauges. As a general principle, sample volumes and number of samples should be kept to a minimum. As a general guide, up to 7.5% of the total blood volume can be taken on a single occasion from a normal, healthy animal on an adequate plane of nutrition with minimal adverse effects; 10% once every two weeks and 15% once every four weeks. For repeat bleeds at shorter intervals, a maximum of 1.0% of an animal's total blood volume can be removed every 24 hours. The acceptable quantity and frequency of blood sampling is dependent on the circulating blood volume of the animal and the red blood cell (RBC) turnover rate (RBC life span of the mouse: 38-47 days). The following must be taken into consideration must be:

- Weight of the animal to be sampled
- Age and health of the animal to be sampled
- Effects of handling stress
- Collection site
- Frequency of sampling necessary
- Training and experience of the personnel performing the collection
- Suitability of sedation and/or anesthesia
- The minimum volume required for analysis. *The maximum permitted blood volume includes blood lost during collection. As a general rule, 20 drops = 1 mL (i.e. 5 drops = 250 uL)*

When collecting blood it is very important that the handler is able to recognize signs of shock and anemia. The combined effect of sample volume and sample frequency without appropriate fluid replacement can cause an animal to go into hypovolaemic shock or become anemic. Packed cell volume, haemoglobin level, red blood cell and reticulocyte counts should be monitored

throughout a series of bleeds using the results from the first sample from each animal as the baseline for the animal.

- Signs of hypovolemic shock include a fast and thready pulse, pale dry mucous membranes, cold skin and extremities, restlessness, hyperventilation, and a sub-normal body temperature.
- Signs of anemia include pale mucous membranes of the conjunctiva or inside the mouth, pale tongue, gums, ears or footpads (non-pigmented animals), intolerance to exercise and with severe anemia, increased respiratory rate when at rest.

If>10% blood volume is required, it is recommended to replace collected blood volume by 3–4 times the volume of blood collected with isotonic fluids (i.e. fluids with same tonicity as blood, such as 0.9% saline or Lactated Ringer's solution).

The Circulating Blood Volume (CBV) of an adult mouse is ~72 ml/kg (0.072ml/g).

To calculate blood collection volumes:
Total Blood Volume (TBV) = Body weight x Circulating Blood Volume

- 1% (maximum) of the TBV can be collected every 24 hours.
- 7.5% (maximum) of the TBV can be collected in a single collection, once per week.
- 10% (maximum) of the TBV can be collected in a single collection, once per every 2 weeks.
- 15% (maximum) of the TBV can be collected in a single collection, once per every 4 weeks.

Example:

- TBV x % (based on desired frequency of collection) = allowable volume to be collected.
- **i.e. For a single collection once per week:** 20 g x 0.072 ml/g = 1.44 ml/g ***then*** 1.44 x 0.075 (7.5% for once per week sample) = 0.1 ml is the maximum allowable volume.

Body Weight (g)	Total Circulating Blood Volume (ml)	Acceptable volume for collection µl (ml)			
		1.0% cumulative or single collection every 24 hrs.	7.5% single collection once per week	10% single collection once per every2weeks	15% single collection onceper every4weeks
15	1.08	11µl	80 (0.08)	108 (0.11)	160 (0.16)
20	1.44	14µl	108 (0.11)	144 (0.14)	216 (0.21)
25	1.80	18µl	135 (0.14)	180 (0.18)	270 (0.27)
30	2.16	22µl	162 (0.16)	216 (0.22)	300 (0.33)

35	2.52	25µl	189 (0.19)	252 (0.25)	375 (0.37)
40	2.88	29µl	216 (0.22)	288 (0.29)	430 (0.43)

This schedule allows for recovery time for the animals as illustrated in the following table:

Percent of blood volume collected in a **SINGLE** sampling	Recovery period (weeks)	Percent of blood volume collected over a 24-HOUR PERIOD (**MULTIPLE** samples)	Recovery period (weeks)
7.5%	1	7.5%	1
10%	2	10 - 15%	2
15%	4	20%	4

2. Materials:

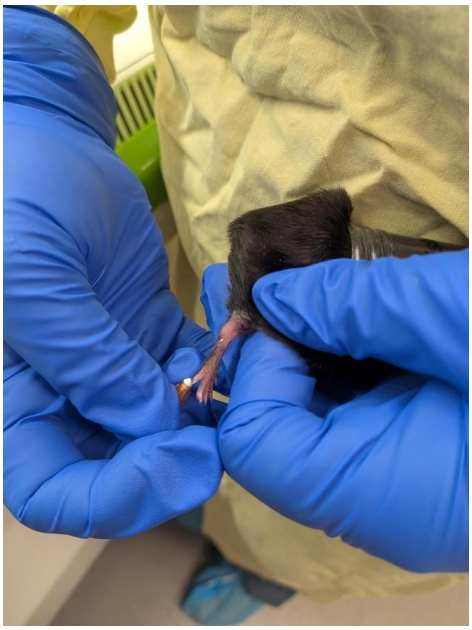
- Restrainers as required
- Sterile needles (multiple sizes ranging from 23-30g)
- Lancets
  - o <20g = 4.0 mm
  - o 20g – 40g = 5.0 mm
  - o >40g = 5.5 mm
- Gauze
- Alcohol swabs
- Petroleum jelly
- Collection tubes
- Sterile swabs
- Clippers (as needed)
- Isotonic fluids such as Lactated Ringers or 0.9% NaCl

3. Procedures:

- The minimal volume required should be collected at all times.
- All collections should be performed by trained and competent individuals.
- The smallest needle size for the collection location (avoiding hemolysis) should be used.
- Each and every animal requires a new sterile syringe and a new sterile needle/lancet. Prepare all equipment in advance.
- Only three attempts per site should be practiced. If unsuccessful, allow another trained person to collect the sample.
- Apply pressure with gauze until hemostasis occurs.

Saphenous Vein

- Each and every animal requires a new sterile needle/lancet.
- If vasodilation assistance is required, heat can be used with the mouse cage for ~ 5 minutes to warm the mouse, making sure the animal does not overheat.
- Place the mouse in a restraining device with hind legs free.
- Remove hair from caudal surface of thigh with clippers (if necessary)
- Swab the site with alcohol.
- Apply petroleum jelly to the collection site. This aids in the formation of a large bead of blood.
- Grasp the fold of skin between the tail and thigh or the skin in front of the femor. The saphenous vein is found on the caudal surface of the thigh.
- Apply pressure to the leg above the knee on the thigh and puncture the vein at a ~45^{O} angle with a 23-25 gauge needle or lancet as shown in *Figure 2*.
- Collect drops of blood as they appear.
- Apply pressure with gauze until hemostasis occurs.
- Monitor mouse for 5-10 minutes to ensure bleeding has stopped.



References:

1) Hoff, Janet, LVT, RLATG. "Methods of Blood Collection in the Mouse", *J. Lab Animal*, Vol 29, No. 010 (November 2000).
2) Kathryn Flynn, NIH - DVR – SoBran
3) The National Center for the Replacement, Refinement, and Reduction of Animals in Research (NC3R's) – Blood Sampling Microsite. http://www.nc3rs.org.uk/bloodsamplingmicrosite/page.asp?id=322

4) http://www.usp.br/bioterio/Artigos/Procedimentos%20experimentais/Bleeding.pdf
5) https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/rodent_bleeding.pdf
6) https://animal.research.uiowa.edu/iacuc-guidelines-blood-collection 7) Regan, et al. JAALAS, 2016. 55 (5):570-576.
8) University of Washington Training Manual
9) PennState Wet Lab for Teaching Common Rodent Techniques
10) Diehl, K.-H. et al., "A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes", *J. Appl. Toxicol.*, **21**, 15–23 (2001)
11) Guidelines for survival bleeding of mice and rats; NIH: http://oacu.od.nih.gov/ARAC/Bleeding.pdf
12) Guide to the Care and Use of Experimental Animals, Vol. 1 (2nd ed), Canadian Council on Animal Care, Canada, 1993
13) Physiological and Pathological Impact of Blood Sampling by Retro-Bulbar Sinus Puncture and Facial Vein Phlebotomy in Laboratory Mice, Teilmann et al., PLOS Published: November 26, 2014 https://doi.org/10.1371/journal.pone.0113225

SOP Revision History:

Date	New Version
September 22,2015	Triennial review
January 25,2018	Triennial review
February 28, 2019	Updated SOP
February 28, 2022	Triennial review
July 18, 2022	Original SOP separated into different blood collections SOPs
June 24, 2026	Triennial review. Cleaned up wording and updated photo