University Animal Care Committee Standard Operating Procedure		
Document No: 10.10	**Subject:** Saphenous Blood Collection in Rats	
Date Issued: July 7, 2011	**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 how to properly collect blood from the saphenous vein.

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 ascertain the most appropriate site for blood collection based on the volume required.

Site	Tail Vein	Saphenous	Cardiac puncture	Jugular
Multiple sampling	Yes	Yes	No	No
Volume	0.05 - 0.1 ml/site	0.1-0.3 ml	1.0-3.0 ml	1.0 ml
Gauge (maximum)	23	25 (23)	23	25 (23)

The following are "good practice" guidelines recommended for blood collection volumes Collection sites and needle gauges.

- The Circulating Blood Volume (CBV) of an adult rat is ~64mL/kg (0.064mL/g).
- 1% (maximum) of the CBV can be collected every 24 hours.
- 7.5% (maximum) of the CBV can be collected in a single collection, once a week.
- 10% (maximum) of the CBV can be collected in a single collection every 2 weeks.
- 15% (maximum) of the CBV can be collected in a single collection every 4 weeks.

To calculate blood collection volumes:

- Body weight x Circulating Blood Volume = Total Blood Volume (TBV)
- TBV x % blood sample required = acceptable volume to be collected
  (i.e. 100g x 0.064mL/g = 6.4mL/g **then** 6.4 x 0.075 = 0.5mL is the max accepted volume)

Body Weight (g)	Total Circulating Blood Volume (mL/g)	Acceptable volume for collection µl (mL)		
		7.5% Single collection/ 1 week	10% single collection/ 2 weeks	15% single collection/ 4 weeks
100	6.4	500 (0.5 ml)	600 (0.6 ml)	900 (0.9 ml)
150	9.6	700 (0.7)	900 (0.9)	1400 (1.4)
200	12.8	900 (0.9)	1200 (1.2)	1900 (1.9)
250	16	1200 (1.2)	1600 (1.6)	2400 (2.4)
300	19.2	1400 (1.4)	1900 (1.9)	2800 (2.8)
350	22.4	1600 (1.6)	2200 (2.2)	3300 (3.3)
400	25.6	1900 (1.9)	2500 (2.5)	3800 (3.8)
450	28.8	2100 (2.1)	2800 (2.8)	4300 (4.3)
500	32	2400 (2.4)	3200 (3.2)	4800 (4.8)

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 anemia.

- 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 increased respiratory rate at rest with severe anemia.
- Packed cell volume, haemoglobin level, red blood cell and reticulocyte counts should be monitored throughout the series of bleeds using the results from the first sample from each animal as the baseline for the animal.
- If volumes larger than10% are collected, replace volumes by 3-4 times the blood volume collected with warmed (30-39 degrees) isotonic fluids.

2. Materials:

- Restrainers as required
- Sterile needles (multiple sizes ranging from 23-30g)
- Sterile gauze
- Alcohol swabs
- Petroleum jelly

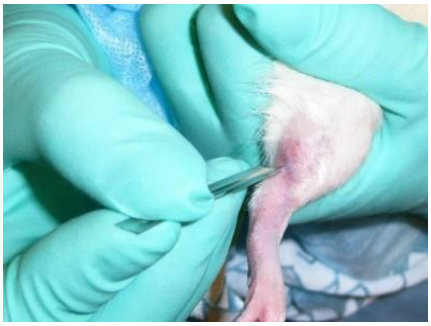
- Collection tubes
- Hair clippers
- Warmed isotonic fluids such as Lactated Ringers or 0.9% NaCl

3. Procedures:

- Only University Animal Care Committee (UACC) approved blood collection techniques can be performed.
- The least volume required should be collected at all times.
- All collections should be performed by trained and competent individuals.
- The smallest needle size that complements collection location without causing hemolysis should be used.
- Each and every animal requires a new sterile syringe and a new sterile needle/lancet.
- Only three attempts per site should be practiced. If unsuccessful, allow another (trained and competent) person to collect the sample.
- Apply pressure with gauze until hemostasis occurs.

Saphenous Vein

- Each and every animal requires a new sterile syringe and a new sterile needle/lancet.
- Prepare your sample tubes and have them readily available.
- If used, apply EMLA cream to the puncture site and wait 15 minutes for it to take effect.
- The saphenous vein lies dorsal then laterally over the tarsal joint and is immediately visible under the skin.
- Remove the animal from the cage and restrain with isoflurane anesthesia (as per SOP 10.6) or by securely wrapping them in a towel.
- Grasp the dorsal end of the thigh and extend the back leg ensuring the leg is not hyper extended (Figure 1). If extended too far, it can impede blood flow. Confirm the animal can breathe comfortably.
- Remove hair with clippers and swab the site with alcohol.
- Apply petroleum jelly over the vein. This aids in the formation of a large bead of blood.
- Using the appropriate gauge needle, puncture the vein at a 45° angle cranially (bevel up). Inserting needle in the caudal direction may damage the sciatic nerve. Allow a bead of blood to form and collect. All blood loss must be included in calculated volumes.
- Apply pressure to the site with gauze until hemostasis occurs.
- Administer supplemental SQ fluids if necessary, depending on the blood volume collected.
- Return the animal to its cage and monitor for any signs of distress.



References:

1) 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)

2) Wolfensohn, S., Lloyd, M. 2nd Edition, Blackwell Science Ltd. 1998.

3) Guidelines for survival bleeding of mice and rats; NIH: http://oacu.od.nih.gov/ARAC/Bleeding.pdf

4) Guide to the Care and Use of Experimental Animals, Vol. 1 (2nd ed), Canadian Council on Animal Care, Canada, 1993: http://ccac.ca/en/CCAC_Programs/Guidelines_Policies/GUIDES/ENGLISH/V1_93/APPEN/APPVIII.HTM

5) The National Centre 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

SOP Revision History:

Date	New Version
January 24, 2012	Triennial review
March 16, 2012	Updated SOP
September 22, 2015	Triennial review
February 28, 2019	Triennial review
February 29. 2022	Triennial review
July 18, 2022	Original SOP separated into different blood collection SOPs