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 retro-orbital 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 / RBC life span of the rat: 42-65 days). Always taken into consideration must be:

- The species to be sampled
- The size of the animal to be sampled
- The age and health of the animal to be sampled
- The effects of handling stress
- The collection site
- The frequency of sampling necessary
- The training and experience of the personnel performing the collection
- The 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, 5% dextrose or Lactated Ringer's solution).

The Circulating Blood Volume (CBV) of an adult mouse is ~72 ml/kg (0.072ml/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 per week.
- 10% (maximum) of the CBV can be collected in a single collection, once per every 2 weeks.
- 15% (maximum) of the CBV can be collected in a single collection, once per every 4 weeks.

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

- 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:

- Gauze
- Collection tubes
- Anaesthetics as required
- Alcaine
- Antibiotic ophthalmic ointment (such as BNP)
- Sterile swabs
- 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 minimal volume required should be collected at all times.
- All collections should be performed by trained and competent individuals.
- Prepare all equipment in advance.
- Only three attempts per site should be practiced. If unsuccessful, allow another trained person to collect the sample.

Retro-Orbital

- Anesthetize mouse as per *SOP 7.6 "Anesthesia in Mice"*.
- Place mouse on table in lateral recumbency.
- Using palm and forefinger of the same hand restrain mouse against table.
- With thumb and forefinger of the same hand, restrain the head and gently open eyelids to expose the eye.
- Alternatively, cradle the mouse in your hand and scruff off to the side which will cause the eyeball to protrude.
- Insert the tube into the medial canthus and hold it at a 60° angle (vertex at canthus).
- Push the tube through the conjunctiva and into the orbital sinus by gently rotating the tube with downward pressure. Changing the angle of the tube may increase the blood flow. The tube passes behind the eye so it should not cause damage. Withdraw the tube after the required amount of blood is obtained. Bleeding usually stops after tube is withdrawn, if not,

apply direct pressure with gauze over closed eye.

- Apply Alcaine and BNP mixture using sterile technique to eye that had been sampled.
- Monitor mouse for 5 to 10 minutes to ensure bleeding has stopped. Recheck in 24 hours.
- Blindness can occur if the optic nerve is damaged as a result of the blood collection tube coming in contact to the nerve which attaches to the middle of the ventral surface of the eye. Ocular ulcerations, puncture wounds, loss of vitreous humor, infection or keratitis may also occur as a result of poor technique.

References:

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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