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 submandibular 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:

- 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
- Collection tubes
- 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.
- 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.

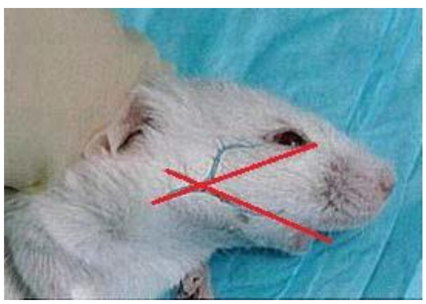
Submandibular

- Each and every animal requires a new sterile needle/lancet. The landmark is the intersection of a line bisecting the rostral canthus of the eye and horizontal line extending the mouth/jaw. *Figure 1.*
- Restrain mouse by scruffing with your thumb and fore finger at the back of the neck, ensuring breathing is not compromised.
- There is a vascular bundle located at the rear of the jawbone. Using the

appropriate lancet size, make a stab into the cheek midway between the ear and the mandible. The correct spot is just above and behind the tip of the mandibular bone, above the dimple.

- Blood droplets will form at the puncture site.
- Use a capillary tube to collect small volumes. Alternatively, using a tube and rack, collect the drops as they fall from the puncture site.
- Use the correct size lancet (as per the manufacturer's guidelines and as described in the Materials section) to ensure the point will not be introduced too deeply into the cheek.
- To stop bleeding, release the scruff. If bleeding continues, apply light pressure with sterile gauze for approximately 5 seconds. Do not scruff the mouse while applying pressure.
- After the mouse is released, it should commence grooming. Mice should be monitored for 5- 10 minutes after the procedure to ensure bleeding has stopped.

According to the NC3Rs, while taking a blood sample from the facial vein is relatively easy technically, the ease of access poses a significant risk of inadvertently sampling too much blood. Additionally, this sampling site has been shown to be stressful for mice and can result in excessive tissue damage (Teilmann et al., 2014), and therefore its application requires that personnel be highly trained.



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SOP Revision History:

Date	New Version
9/22/2015	Triennial review
1/25/2018	Triennial review
2/28/2019	Updated SOP
2/28/2022	Triennial review
7/18/2022	Original SOP separated into different blood collections SOPs