***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 submental 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
- Petroleum jelly
- Collection tubes
- Sterile swabs
- Clippers
- 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.

**Submental**

- Restrain the mouse using the scruff technique. It is important to have a secure hold, with the head elevated extending the neck region.
- Ensure the skin is taut without restricting breathing.

- Landmark the submental veins – in white background animals the convergence of facial and submental veins create a dark area under caudal to the lower jaw. Application of alcohol to this site may help to visualize. *Figure 3.*
- When not readily visible, move slightly rostro lateral from the group of hairs located on the midline of the throat. The target site will be a slightly softer spot in the tissue just medial to the facial vein.
- Once the animal is restrained and the puncture site determined, swab the site with alcohol.
- Use the 4-5mm lancet, or the 25-27g needle to puncture the dark area, just medial to the jaw on each side. Insert and withdraw in a smooth, firm fashion. The lancet should be held at a slight angle to the animal (~10-15°). The blood should flow freely. *Figure 4.*
- Collect blood into capillary tubes, or directly into collection vials.
- Bleeding should stop upon release of the scruff.
- Monitor mouse for 5-10 minutes to ensure bleeding has stopped. If necessary, apply pressure with gauze until hemostasis occurs.

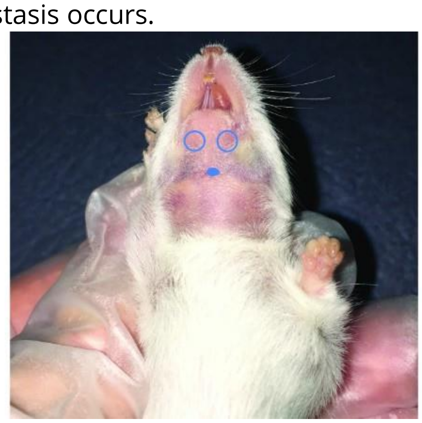

**Figure 3.** The darker areas indicate vessels under the skin, blue dot indicates the location of the hair or fur whorl. Circled areas indicate the approximate sites where the facial and submental veins converge. Neck shaved for photography. Image from: Regan, et al. JAALAS, 2016. 55 (5):570-576.


**Figure 4.** University of Washington, courtesy of Erika French.

## *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) 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 |
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