University Animal Care Committee Standard Operating Procedure		
Document No: 7.10	**Subject:** Blood Collection in Mice	
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Location: Queen's University

Responsibility: Principal Investigators (PI), Research Staff, Veterinary Staff

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe these methods of blood collection in mice: submandibular, saphenous, retro-orbital, submental, tail vein, and cardiac puncture.

1. Introduction and Definitions:

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:

- Heat lamp
- Restrainers as required
- Sterile syringes
- Sterile needles (multiple sizes ranging from 23-30g)
- Sterile scalpel blades
- Lancets
  - <20g = 4.0 mm
  - 20g – 40g = 5.0 mm
  - >40g = 5.5 mm
- Gauze
- EMLA cream*****
- Alcohol swabs
- Petroleum jelly
- Collection tubes
- Anaesthetics as required
- Alcaine
- Antibiotic ophthalmic ointment (such as BNP)
- Sterile swabs
- Cautery pen
- Clippers
- Isotonic fluids such as Lactated Ringers or 0.9% NaCl

***** EMLA cream requires a minimum 15 minute absorption time post application (species and site dependent). It is strongly recommended to use this topical anaesthetic prior to any injection (particularly in the case of novice handlers), however this step may be waived if it contributes to an animal's stress level and/or is impractical in its application.

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.

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

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.

Saphenous Vein

- Each and every animal requires a new sterile needle/lancet.
- If vasodilation assistance is required, place a heat lamp over the occupied 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.
- Swab the site with alcohol.
- If used, apply EMLA cream to the puncture site and wait 15min for it to take effect.
- Return mouse to restraining tube and apply petroleum jelly to the collection site. This aids in the formation of a large bead of blood.

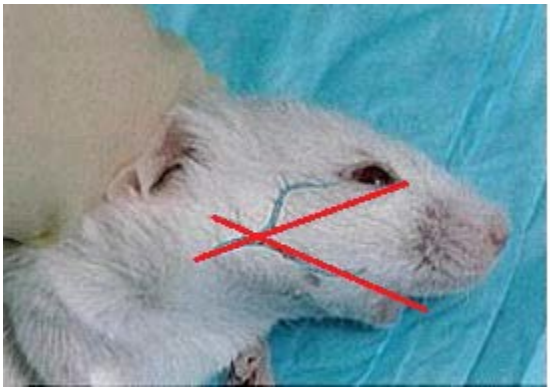

Figure 1

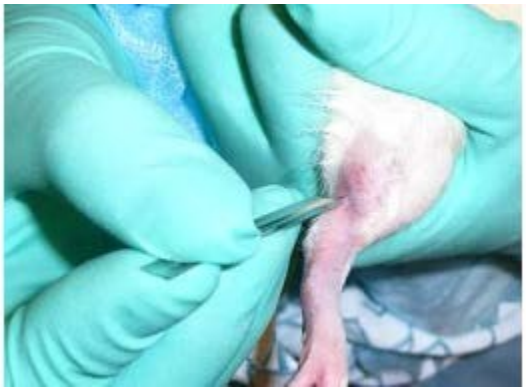

Figure 2

- Grasp the fold of skin between the tail and thigh. 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.

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.

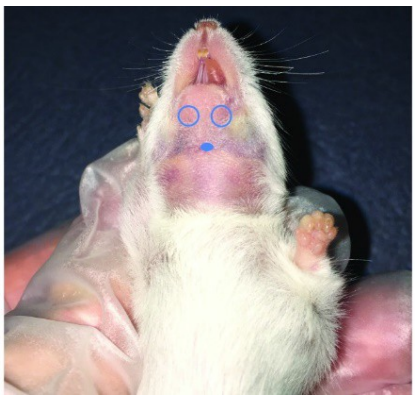

Figure 3. The darker areas indicate vessels under the skin, blue dot indicates the location of the hair or fur whorl. Circled areas indicate the approximate sites where the facial and submental veins converge. Neck shaved for photography. Image from: Regan, et al. JAALAS, 2016. 55 (5):570-576.

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

Tail Vein (intravenous)

- Each and every animal requires a new sterile needle/lancet.
- Place mouse in restraining tube.
- Swab the tail with alcohol.
- Using a heat lamp, direct the heat only to the tail for 5-10 seconds for vasodilation.
- Hold tail and locate lateral tail veins, refer to *Figure 5*.
- Insert 25 gauge needle at a 10^{o} angle to puncture the vein.
- For serial collections, a small nick in the tip of the tail using a sharp, sterile scalpel blade is permitted. This technique works well for clot dislodgment and repeat sampling. Hemostasis must be ensured; a cautery pen may be used post-collection, if needed.
- Withdraw needle and collect blood drops in capillary tube.
- Apply pressure with gauze until hemostasis occurs.

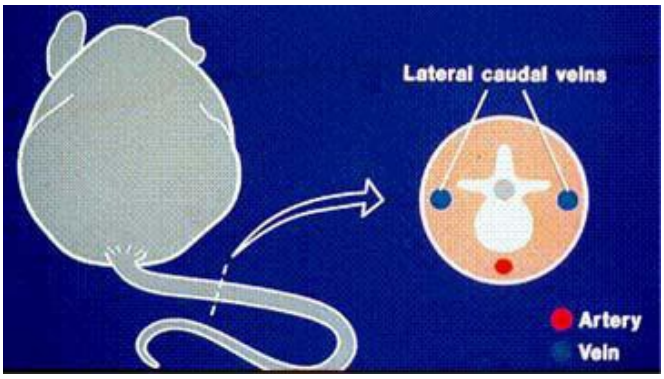

Figure 5.

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° – 90° 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 both eyes.
- 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.

Cardiac Puncture

- Each and every animal requires a new sterile syringe and a new sterile needle/lancet. This is a terminal procedure.
- There are three methods. Dorsal recumbency (lateral and ventral aspirate) and lateral recumbency.
- Anesthetize the mouse following the *SOP 7.6 "Anaesthesia in Mice"*.
- When sterility is of concern, the skin surrounding the desired puncture site can be excised to expose the underlying muscle area for needle insertion.
- Once the animal has reached a surgical plane of anaesthesia, lay the animal either in dorsal recumbency or lateral recumbency.
- Release the vacuum on the syringe prior to skin puncture.

Dorsal Recumbency (Lateral aspirate)

- Using the elbow to help indicate location along the rib cage, palpate for a strong heartbeat.
- Insert the needle bevel up into the thoracic cavity at 15-20° angle directly lateral to the midline.
- When there is evidence of blood within the hub of the needle, steady the syringe and withdraw blood slowly.

Dorsal Recumbency (Ventral aspirate)

- From the sternum trace down the centre of the ribcage and locate the xyphoid process of the rib cage. Allow your needle to "fall" below this landmark.
- Insert the needle bevel up into the sternum and angle the syringe approximately 30 degrees cranially or towards the strongest heartbeat.
- When there is evidence of blood within the hub of the needle, steady the syringe and withdraw blood slowly.

Lateral Recumbency

- Place the animal on its right side facing down.
- Using the elbow to help indicate location along the rib cage, palpate for a strong heartbeat.
- Insert the needle into the thoracic cavity where the heart beat is the strongest.
- When there is evidence of blood within the hub of the needle, steady the syringe and withdraw blood slowly.

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