

UBC ANIMAL CARE COMMITTEE

TECH 06a – Blood Collection from the Lateral or Medial Saphenous Veins in the Adult Mouse SOP

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Version No. 2

PURPOSE:

- To describe the procedure for performing blood collection from the lateral or medial saphenous veins in adult mice.
- This Standard Operating Procedure (SOP) follows the Canadian Council on Animal Care (CCAC) current guidelines for acceptable blood collection volumes and collection sites in rodents.

RESPONSIBILITY:

- All those trained persons listed on an approved Animal Care Committee (ACC) Animal Use Protocol who are responsible for performing blood collection.
- All animal users performing blood collection in rodents must have successfully completed the UBC Animal Care Services (or equivalent) Introduction to Working with Rodents in Research (IWRR) and Rodent Restraint/SQ/IP injections (RSCIP) courses.

MATERIALS: *(can be purchased from Animal Care Services)*

- Fur clippers or depilatory cream (i.e. Nair® for Sensitive Skin)
- 70% Isopropyl alcohol
- Cotton tipped applicators
- Petroleum jelly (i.e. Vaseline®)
- Blood collection tubes
 - Microhematocrit capillary tube, microcentrifuge tube, pipette tip, etc.
 - With or without additives depending on final analysis of blood
 - i.e. EDTA, heparin, etc.
- 25-26G Needle – sterile, one per animal
- 2"x2" gauze
- Appropriately sized restrainer (if applicable)
- Sharps container



Table 1 - BLOOD COLLECTION VOLUME LIMITS AND MINIMUM RE-SAMPLING TIME PERIODS IN MICE¹

| Species: Mouse | Needle Gauge | Single blood sample | | Multiple blood samples ² | |
|---|--------------|--------------------------------|---|--|---|
| | | Maximum % blood volume removed | Required recovery period before additional blood sampling | Maximum % blood volume removed within 24-hours | Required recovery period before additional blood sampling |
| Estimated Total Blood Volume 65ml/kg (60-70ml/kg) | 25-26G | 7.5% | 1 week | 1% | 24 hours |
| | | 10% | 2 weeks | 7.5% | 1 week |
| | | 15-20% | 4 weeks | 10-15% | 2 weeks |
| | | | | 20% | 4 weeks |
| | | | | | |

¹ The total blood volume assumes that the mouse is a healthy adult that is normally hydrated and non-obese. Greater than the recommended volumes or frequency of blood sampling should not be performed unless justified

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and approved on the Animal Care Protocol and increased monitoring for complications implemented. For blood samples 15% or greater, 20 ml/kg of subcutaneous fluids (Lactated Ringers' Solution) should be administered to prevent hypovolemia. See important notes at the end of this SOP.

² When multiple blood samples are collected over a period of time, the total sample volume is reduced because it takes time for hematological parameters to return to normal. The more frequent the sampling, the smaller the volumes permitted.

PROCEDURE:

1. Gather all supplies and required equipment.
2. Weigh the animal and calculate the volume of blood that can safely be collected based on the frequency of collection (refer to Table 1 for maximum recommended volumes and Page 8 for how to calculate volume).
3. Choose the restraint method based on whether the medial or lateral saphenous vein will be used.

Manual Restraint – most commonly used for medial saphenous vein blood collection

4. “Scruff” (gather the skin) the mouse with your non-dominant hand.
5. Use middle or ring finger of your non-dominant hand to gently squeeze the skin on the upper, inner thigh just above the knee (see Figure 1).
6. A proper restraint will ensure the leg is extended, the animal cannot bend its knee or pull its leg close to its body, and will occlude the vein.

Figure 1



Restrainer – used for medial or lateral saphenous vein blood collection (See Appendix 1 for different types of restrainers)

7. Choose a restrainer that:
 - a. Has breathing holes
 - b. Has no sharp edges
 - c. Is the correct size for the animal
 - i. The mouse cannot turn its head over its shoulder
 - ii. The mouse can breathe freely
8. Place mouse in the restrainer and with your non-dominant hand, gently grasp the loose skin just above the upper thigh (see Figures 2a and 2b).

- a. A proper restraint will ensure the leg is extended, the animal cannot bend its knee or pull its leg close to its body, and will occlude the vein.

Figure 2a: Falcon tube restraint for medial saphenous vein



Figure 2b: Falcon tube restraint for lateral saphenous vein



- b. If the restrainer is too long, placing a piece of paper towel at the far end will prevent the mouse from fully entering the restrainer so the hind legs can be accessed. Ensure the paper towel does not block the breathing holes (see Figure 3).

Figure 3



Blood Collection

9. Locate the medial (inner) or lateral (outer) saphenous vein (see Images 4a and 4b and Figures 4c and 4d).

Image 4a Medial Saphenous Vein

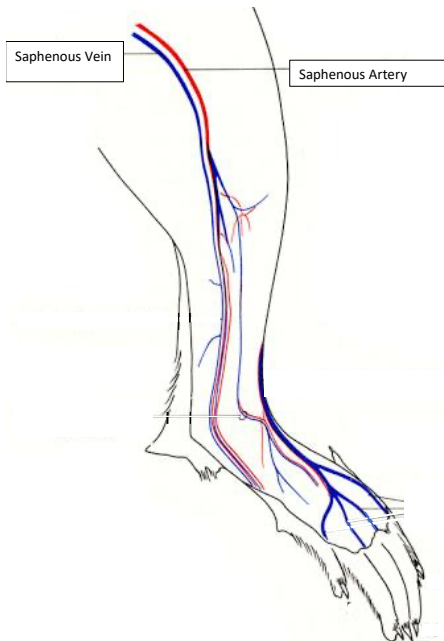


Image 4b Lateral Saphenous Vein



Figure 4c Medial Saphenous Vein



Figure 4d Lateral Saphenous Vein



10. Remove the hair over the saphenous vein.
 - a. Fur clippers (see Figure 4).
 - i. Keep the blades of the clippers parallel to the skin
 - ii. Do not apply downward pressure to avoid cutting the skin.
 - iii. Shave the fur in the opposite direction of hair growth.

Figure 4



- b. Depilatory cream (i.e. Nair® for Sensitive Skin).
 - i. Apply a thin layer of cream onto the fur overlying the saphenous vein using a cotton-tipped applicator and gently rub so the cream contacts the skin (see Figure 5a).
 - ii. DO NOT leave in contact with the skin for more than 30 seconds since the cream can cause chemical burns.
 - iii. Remove all cream and loosened hair with a gauze or cotton-tipped applicator moistened in water or saline (see Figure 5b), not alcohol.
 - iv. It is critical to remove all depilatory cream residue to prevent chemical burns of the skin.

Figure 5a Medial Saphenous Vein

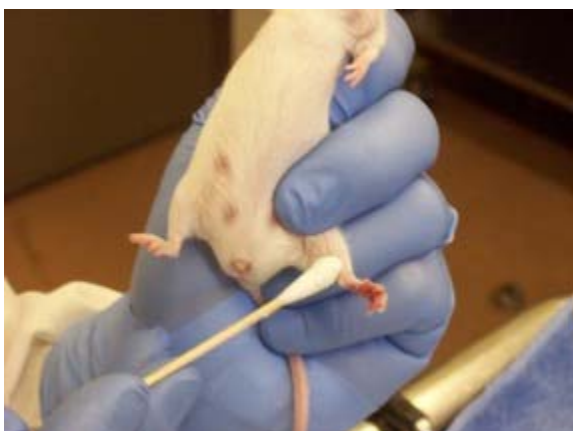


Figure 5b Medial Saphenous Vein



- 11. Swab the planned puncture area with a cotton-tipped applicator moistened with 70% isopropyl alcohol to help visualize the vein (see Figures 6a and b).

Figure 6a: Medial Saphenous Vein



Figure 6b: Lateral Saphenous Vein



12. Apply a thin film of Vaseline® to the site with a cotton-tipped applicator (see Figures 7a and b).
- This will help make collection easier as the blood will form a “bubble” on the surface of the skin and not run down the leg.

Figure 7a Medial Saphenous Vein

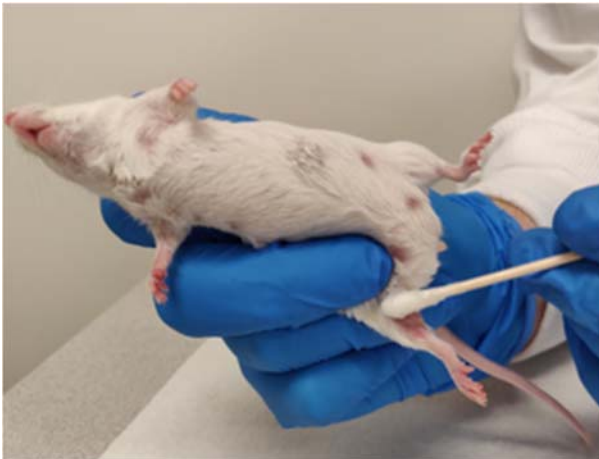


Figure 7b Lateral Saphenous Vein



13. Use a sterile 25-26G needle to puncture the blood vessel perpendicular to the skin at the most proximal (closest to the body) visible area of the vein (see Figures 8a and 8b).
- Leave the needle in the vein for 2-3 seconds to allow the skin to stretch around the puncture site which will allow the blood to exit the puncture site and minimize bruising.
 - Do not insert the needle past the bevel of the needle (1-2 mm deep) to avoid damaging underlying structures.
 - Place the needle in a sharps container.

Figure 8a Medial Saphenous Vein

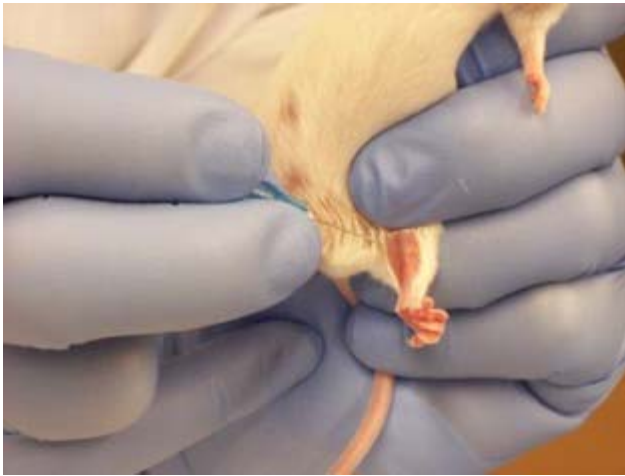
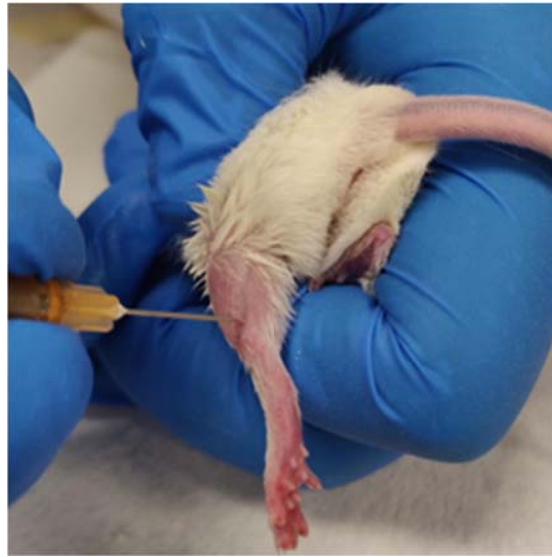


Figure 8b Lateral Saphenous Vein



14. Collect the blood into the desired collection tube (see Figures 9a and b).
 - a. Do not collect more than the calculated amount for the planned collection frequency.
 - b. The blood may exit the vein quickly so be prepared to collect the first drops as this volume will count towards the total volume that can be collected.
 - c. Do not touch the skin or the puncture site with the collection tube as this can cause the blood to clot.
 - d. Hold the collection tube just under the puncture site with the far end of the collection tube below the puncture site so gravity helps fill the tube.
 - e. If bleeding slows or stops, gently bend the ankle repeatedly to “pump” the blood from the foot.

Figure 9a Medial saphenous vein



Figure 9b Lateral saphenous vein



15. Once the desired volume of blood is collected, apply gentle pressure over the puncture site for 60 seconds with a dry gauze or cotton tipped applicator without losing control of the leg (see Figures 10a and 10b).

Figure 10a Medial saphenous vein

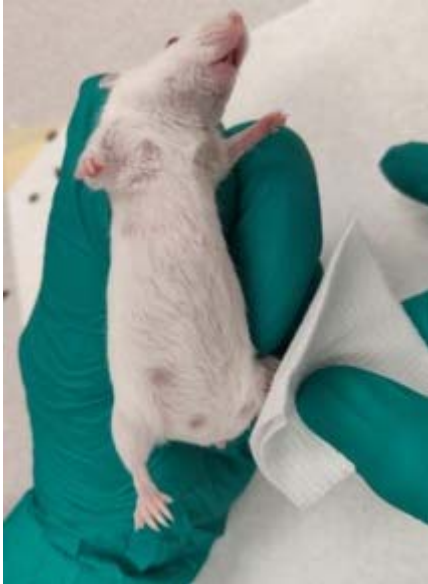


Figure 10b Lateral saphenous vein



16. Slowly lift the gauze or cotton tipped applicator to check that the bleeding has stopped. If bleeding starts again, place gauze over puncture site for an additional 60 seconds as above.
17. Once bleeding has stopped, place the mouse back in its cage and monitor for 5-10 minutes to ensure hemostasis (bleeding has stopped). It is not uncommon for the mouse to start bleeding again once it is active in its cage.
18. If bleeding recurs, remove mouse from the cage and put pressure on the puncture site for one minute. Make note of total estimated blood loss since this may impact when the next samples can be collected.
19. For repeat samples, a new puncture site can be made distal to the previous site (towards the foot) or the other vein (lateral or medial saphenous) can be used.
20. Note procedure (location of blood collection and volume) and date taken on cage card/monitoring records.

CALCULATING VOLUME (IN ML) THAT CAN BE COLLECTED:

- Convert animal's weight from grams to kilograms
 - Divide the weight in grams by 1000
 - E.g. 25g mouse \div 1000 = 0.025kg
- Calculate the total volume that can be collected in ml for a given collection frequency
 - Total volume allowed to be collected
= blood volume (ml/kg) x weight of animal (kg) x % to be collected based on collection frequency

- **E.g. Single blood collection every 2 weeks on a 25g (0.025kg) mouse**
 - Blood volume = $65\text{ml/kg} = 65\text{ ml/kg} \times 0.025\text{kg} = 1.6\text{ ml}$
 - 10% of total blood volume can be collected every 2 weeks = $10/100 = 0.1$
 - **Total volume allowed** = blood volume of mouse x % allowed to be collected = $1.6\text{ ml} \times 0.1 = \mathbf{0.16\text{ ml of blood every 2 weeks}}$

- **E.g. Multiple blood collections over 8 hours in a 25g (0.025kg) mouse, repeated in 4 weeks**
 - Blood volume = $65\text{ml/kg} = 65\text{ ml/kg} \times 0.025\text{kg} = 1.6\text{ ml}$
 - 20% of total blood volume over multiple time points can be collected during this 8-hour period as the next sampling period is in 4 weeks = $20/100 = 0.2$
 - **Total volume allowed** = blood volume of mouse x % allowed to be collected = $1.6\text{ ml} \times 0.2 = \mathbf{0.32\text{ ml of blood every 4 weeks}}$
→ this is the total volume that can be collected within a 24 hour period (in this example, only over 8-hours).
 - **Volume permitted at each time point = total volume ÷ # of time points** (within 24 hours)
E.g. if collecting at 6 times points during this 8-hour period
= $0.32 \div 6 = 0.05\text{ ml}$ can be collected at each time point
E.g. if collecting at 2 time points during this 8-hour period,
= $0.32\text{ ml} \div 2 = 0.16\text{ ml}$ can be collected at each time point

IMPORTANT NOTES:

- This method of blood collection can reliably collect up to 100 ul of blood.
- The total volume of blood collected includes desired volume of blood plus any blood loss (i.e. on gauze or cotton tipped applicators or bruising).
- Collection of greater than the recommended volume of blood will lead to anemia which will:
 - Decrease blood circulation to vital organs
 - Change how drugs are absorbed, distributed, metabolized and excreted
 - Can lead to cardiovascular collapse, hypovolemic shock and death.
- Fluid replacement in the form of warmed sterile Lactated Ringers Solution or 0.9% Normal Saline administered subcutaneously at a volume of 20 ml/kg should be given to prevent hypovolemia if 15% or greater blood sample volumes are collected.
- Minimize the time the animal is restrained.
 - Ensure the animal can breathe normally and there are an adequate number of air holes when using a restrainer.
 - Ensure the animal cannot turn its head over its shoulder in the restrainer since it can get trapped and obstruct breathing
 - Monitor for signs of distress or overheating (see complications below)
- Clean and dry the restrainer between each animal.
- Ensure that the leg of the animal is held in a normal position to avoid damage to the muscles, tendons, ligaments or bones of the animal.
- A new sterile needle should be used for each animal.

- If the first puncture is not successful for collection of the required volume, an additional puncture of the same vein or a second vein is permitted.

COMPLICATIONS:

- **Damage to the skin caused by fur clippers or depilatory cream:**
 - **Cause:** Fur clippers being pushed too hard against the skin or not being held parallel to the skin and causing a laceration. Depilatory cream can cause chemical burns to the skin if left on too long or not completely removed.
 - **Clinical signs:**
 - Laceration (cut)
 - Pain, redness
 - Ulceration of skin (can happen up to 48 hours later)
 - **Response:** Contact your Clinical Veterinarian for treatment options, which may include analgesics, antibiotics and/or topical treatment of any wounds. Severe wounds or necrosis exposing underlying muscle will require euthanasia.
- **Injury or distress caused by restrainer**
 - **Cause:** Restrainer which is too small or not enough air holes. Occasionally an injury when the animal struggles to escape.
 - **Clinical signs:**
 - Feet bright red (overheating) or blue colour (animal cannot breathe)
 - Open mouth breathing
 - Bulging eyes
 - Tongue protruding from mouth or tongue appears blue/purple
 - Torn toenails or broken teeth
 - **Response:** Immediately release the animal from the restrainer and return it to a cage. If animal is conscious and moving around, monitor for 30 minutes before reattempting restraint with a different method. If animal is open mouth breathing, provide supplemental oxygen. If there is any bleeding, apply direct pressure until it has stopped. Contact your Clinical Veterinarian for treatment options if the animal is physically injured or not returning to normal behaviour or activity within 30 minutes. Do not use the same restrainer again for this animal.
- **Continued blood loss:**
 - **Causes:** Direct pressure has not been held on the puncture site long enough, the animal has a clotting abnormality, or the occlusion on the vessel is causing increased pressure.
 - **Clinical signs:**
 - Continued bleeding or rebleeding at the puncture site.

Response: Immediately remove animal from its cage and apply direct pressure to the site of bleeding with a dry gauze for at least 60 seconds before checking that the bleeding has ceased. Continue re-applying pressure for 60 second intervals until bleeding has stopped. Hemostasis may take up to a few minutes, depending on the animal's blood pressure and clotting ability. The longer you can apply pressure without stressing the mouse, the better it will clot. If more than 3-4 drops of blood were lost, administer 20 ml/kg of SQ Lactated Ringer's or 0.9% Normal Saline and monitor the colour of the extremities and behaviour. If animal's extremities are pale or the animal appears weak or lethargic, contact your Clinical Veterinarian. If

greater than 20% of the total blood volume has been lost, further blood collection must not occur for at least 4 weeks. Record total blood loss on monitoring records.

- **Bruising of leg around site of puncture:**
 - **Cause:** blood seeped under the skin either because the animal moved its leg during collection, direct pressure was not applied quickly enough after collection or bleeding restarted after sample collection.
 - **Clinical signs:** Purple discolouration of skin
Response: Bruising is typically self-limiting but extensive bruising that prevents visualization of the vein will prevent further blood collection from that site until the bruising resolves. Provide analgesia if mouse is limping or licking at the area. Contact the Clinical Veterinarian if bruising is not resolving within 2-3 days.
- **Lesion formation at injection site:**
 - **Cause:** Infection or self-mutilation of puncture site. Can develop 2-5 days after blood collection.
 - **Clinical signs:** A lesion or wound can form if the puncture site becomes infected, depilatory cream irritated the skin or if the animal begins to chew at the area.
 - Pain (chewing, scratching at site, lameness-see below)
 - Redness
 - Infection (moist, purulent discharge)**Response:** Monitor at least once a day. Applying topical antibiotics as soon as redness is seen can help reduce the formation of a lesion or infection. Hibitane or Polysporin creams are good first choices. Contact your Clinical Veterinarian for further treatment options which may include analgesics, antibiotics and/or topical treatments of any wounds. Severe wounds or necrosis will require euthanasia.
- **Lameness or limping:**
 - **Cause:** The leg or foot was damaged by being held in an abnormal position; a peripheral nerve was damaged during the collection; or swelling/bruising of the area is causing pain.
 - **Clinical signs:**
 - Limping
 - Not using leg (non-weight bearing)
 - Chewing at leg/foot
 - Less willing to move around cage
 - Weight loss
 - **Response:** Monitor at least once a day and provide analgesics for 2-3 days. If limping/lameness continues beyond 2-3 days or analgesics are not effective, contact your Clinical Veterinarian.
- **Anemia (pale extremities):**
 - **Cause:** Greater than the maximum amount of blood has been lost/collected within a certain time period. This can happen through:

- Difficulty stopping the bleeding after collection
- Collecting too much blood or too frequently
- Bruising (loss of blood under the skin)
- Rebleeding after the animal is returned to the cage
- **Clinical signs:**
 - Pale extremities (ears, feet, eyes, genitals)
 - Animal appears weak, wobbly when walking or lethargic
 - Slower or deeper breathing than normal
- **Response:** Stop all further blood collection and contact your Clinical Veterinarian for treatment options.

REFERENCES:

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Appendix 1 -Restrainers

