UBC ANIMAL CARE COMMITTEE
TECH 06b – Blood Collection from the Lateral or Medial Saphenous Veins in the Adult Rat SOP

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PURPOSE:

- To describe the procedure for performing blood collection from the lateral or medial saphenous veins in adult rats.
- 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
- 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.
- 23-25g 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 RATS[1]

Species: Rat	**Needle Gauge**	**Single blood sample**		**Multiple blood samples [2]**	
Estimated Total Blood Volume 65ml/kg (60-70ml/kg)	23-25 G	**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
		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 rat 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 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 volume and Page 7 for how to calculate volume).
3. Choose the restraint method based on whether the medial or lateral saphenous vein will be used and how calm the rat is. Calm rats can be restrained against the front of your body as shown in the following pictures while nervous rats can be "Burrito wrapped" (See Appendix 1 for Burrito wrapping instructions).

Lateral Saphenous Vein – restraint of hind leg

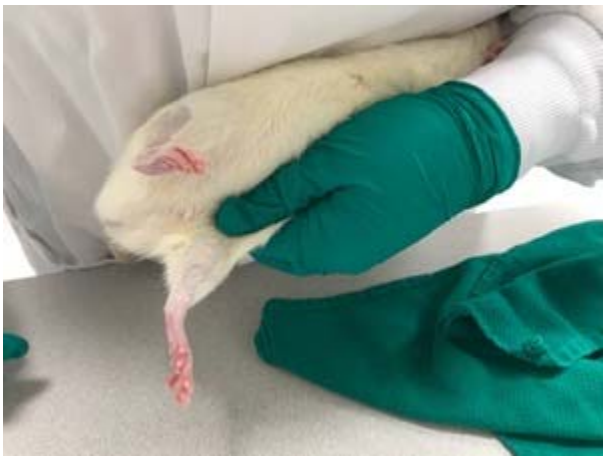
4. With your non-dominant hand, and the rat tucked against your body, gently grasp the rat's loose skin between your thumb and forefinger just above the rat's upper thigh of the leg farthest from your body (see Figure 1).
    a. A proper restraint will ensure the leg is extended, the animal cannot bend its knee and pull its leg close to its body, and will occlude the vein.

Figure 1

Medial Saphenous Vein – restraint of hind leg

5. With your non-dominant hand, and the rat tucked against your body, gently grasp the rat's loose skin between your thumb and forefinger just above the rat's upper thigh of the leg closest to your body (see Figure 2).
    a. A proper restraint will ensure the leg is extended, the animal cannot bend its knee and pull its leg close to its body, and will occlude the vein.

Figure 2



Blood Collection

6. Locate the Lateral (outer) or medial (inner) saphenous vein (see Images 3a and 3b and Figures 3c and 3d).

Image 3a Lateral Saphenous Vein

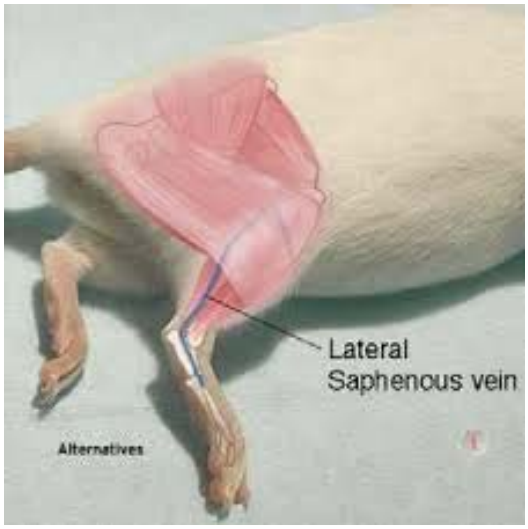


Image 3b Medial Saphenous Vein

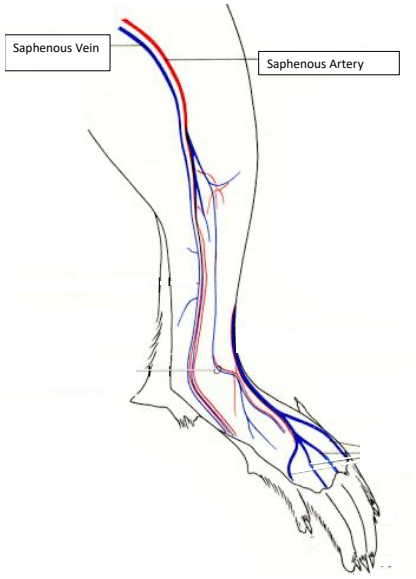


Figure 3c Lateral Saphenous Vein

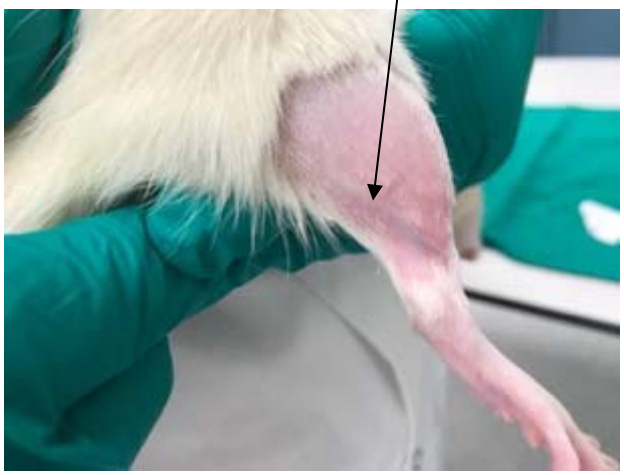
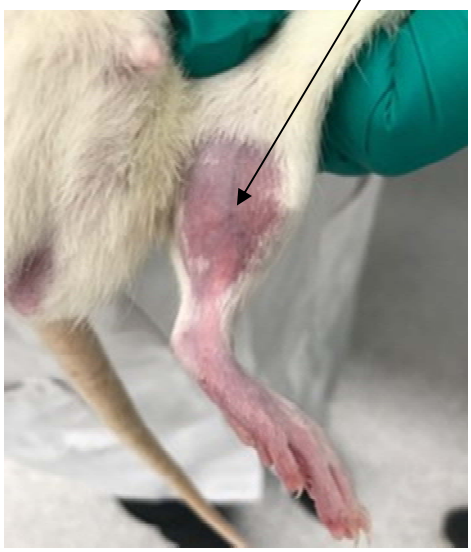
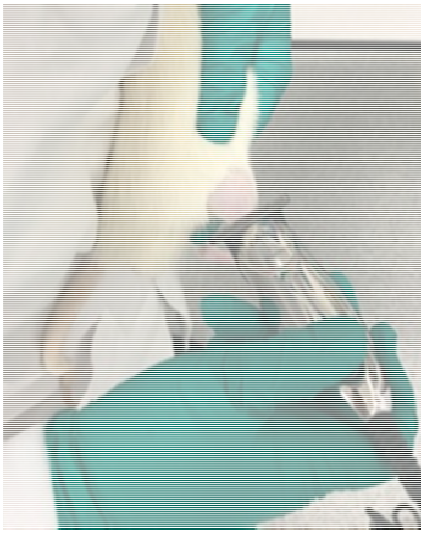


Figure 3d Medial Saphenous Vein



7. Remove the hair over the saphenous vein with fur clippers (see Figure 4).
    a. Keep the blades of the clippers parallel to the skin.
    b. Do not apply downward pressure to avoid cutting the skin.
    c. Shave the fur in the opposite direction of hair growth.

Figure 4



Depilatory cream (i.e. Nair® for Sensitive Skin) is not recommended for removing rat fur since it takes longer than 30 seconds to remove rat fur and can cause a chemical burn. If using, follow these important steps:

a. 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.
b. DO NOT leave in contact with the skin for more than 30 seconds since the cream can cause chemical burns.
c. Remove all cream and loosened hair with a gauze or cotton-tipped applicator moistened in water or saline (see Figure 5b), not alcohol.

d. It is critical to remove all depilatory cream residue to prevent chemical burns of the skin.

8. Swab the planned puncture area with a cotton-tipped applicator moistened with 70% isopropyl alcohol to help visualize the vein (See Figures 5a and 5 b).

Figure 5a Lateral Saphenous Vein

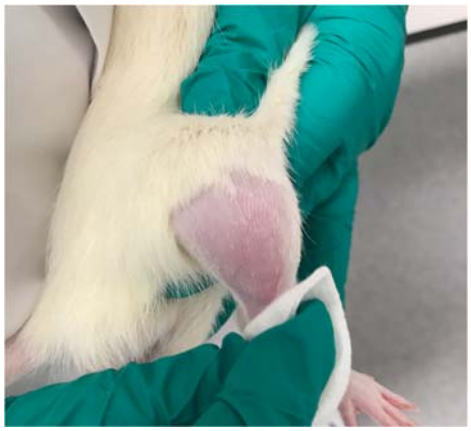


Figure 5b Medial Saphenous Vein

9. Apply a thin film of Vaseline® to the site with a cotton-tipped applicator (see Figures 6a and 6b).
    a. 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 6a Lateral Saphenous Vein

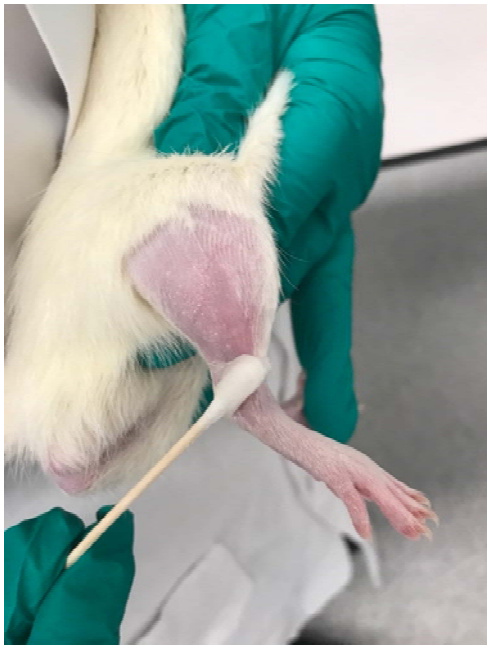


Figure 6b Medial Saphenous Vein

10. Use a sterile 23-25G 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 7a and 7b).

a. 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.
b. Do not insert the needle past the bevel of the needle (1-2 mm deep) to avoid damaging underlying structures.
c. Place the needle in a sharps container.

Figure 7a Lateral Saphenous Vein

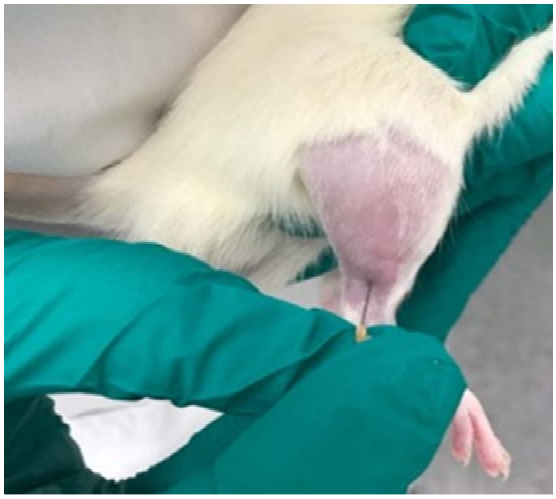
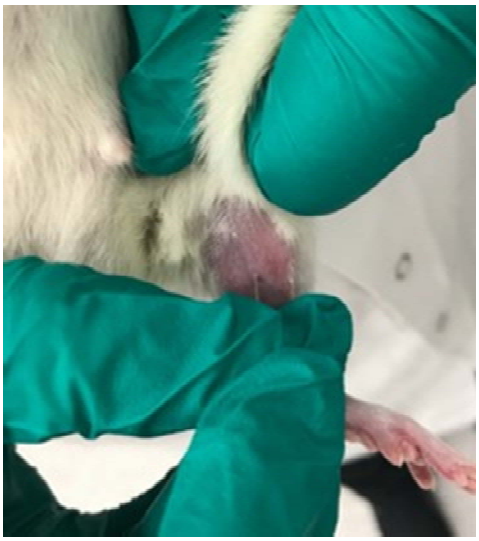


Figure 7b Medial Saphenous Vein



11. Collect the blood into the desired collection tube (see Figures 8a and 8b).
    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 foot repeatedly to "pump" the blood from the foot.

Figure 8a – Lateral saphenous vein

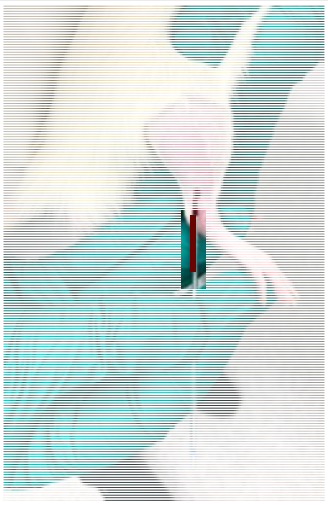
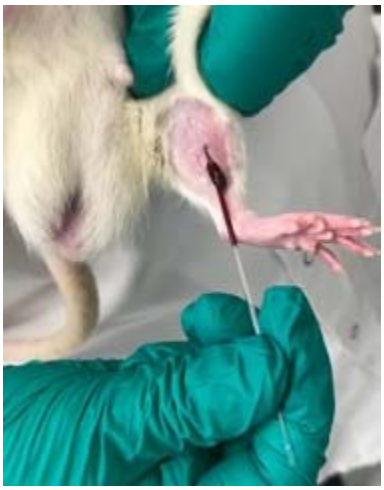


Figure 8b – Medial saphenous vein



12. 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 9a and 9b).

Figure 9a – Lateral saphenous vein

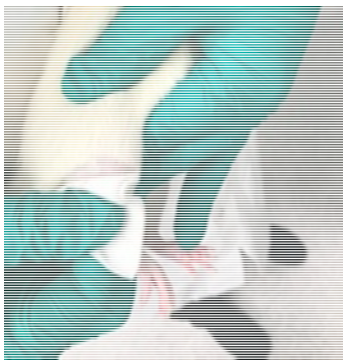


Figure 9a – Medial saphenous vein

13. Slowly lift the gauze to check that the bleeding has stopped. If bleeding starts again, place gauze over puncture site for an additional 60 seconds as above.
14. Once bleeding has stopped, place the rat back in its cage and monitor for 5-10 minutes to ensure hemostasis (bleeding has stopped). It is not uncommon for the rat to start bleeding again once it is active in its cage.
15. If bleeding recurs, remove the rat 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.
16. For repeat samples, a new puncture site can be made distal to the previous site (towards the foot) or use the opposite vein (lateral or medial saphenous).
17. Note procedure (date, location of blood collection and volume collected) 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. 250g rat ÷ 1000 = 0.25kg
- Calculate the 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 250g (0.25 kg) rat**
    - Blood volume = 65ml/kg = 65 ml/kg x 0.25kg = 16.25 ml
    - 10% of total blood volume can be collected every 2 weeks = 10/100 = 0.1
    - **Total volume allowed** = blood volume of rat x % allowed to be collected = 16.25 ml x 0.1 = **1.6 ml of blood every 2 weeks**
  - **E.g. Multiple blood collections over 8 hours in a 250g (0.25kg) rat, repeated in 4 weeks)**
    - Blood volume = 65ml/kg = 65 ml/kg x 0.25kg = 16.25 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 rat x % allowed to be collected = 16.25 ml x 0.2 = **3.2 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
  = 3.2 ÷ 6 = 0.53 ml can be collected at each time point

  E.g. if collecting at 2 time points during this 8-hour period,
  = 3.2 ml ÷ 2 = 1.6 ml can be collected at each time point

IMPORTANT NOTES:

- This method of blood collection can reliably collect up to 200 ul of blood.
- The total volume of blood collected includes desired volume of blood collected 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
  - Monitor for signs of distress or overheating
- Use a new towel for each animal.
- Ensure the leg of the animal is held in a relaxed and 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**:
  - **Cause:** Fur clippers being pushed too hard against the skin or not being held parallel to the skin and causing a laceration.
  - **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 restraint**
  - **Cause:** Method of restraint does not allow enough air passage (towel weave is too tight or animal is held too tightly restricting chest movement) or animal is overheating. 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 restraint 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.

- **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 rat, 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 <u>at least</u> 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 rat is limping or licking at the area. Contact the Clinical Veterinarian if bruising has not resolved 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 solution 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:

- Removal of Blood from Laboratory Mammals and Birds. Laboratory Animals (1993) 27, 1-22
  http://onlinelibrary.wiley.com/doi/10.1002/jat.727/abstract

- UBC Animal Care Committee Policy 006: Acceptable Methods of Rodent Blood Withdrawal (https://animalcare.ubc.ca/sites/default/files/documents/006_Rodent_blood_withdrawal.pdf)
- A good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes; Diehl, K et al. 2001 http://onlinelibrary.wiley.com/doi/10.1002/jat.727/abstract
- Skin turgor as a quantitative index of dehydration in rats. Pediatrics: 19: 810-815. Laron, Z., and Crawford J 1957. http://www.ncbi.nlm.nih.gov/pubmed/13431305

APPENDIX 1 : Towel wrapping ("burrito wrap")

a. Ensure that the weave of the towel allows the rat to breathe once wrapped and that it is not so loose a weave that the rat can catch and tear its toenails.
    a. Avoid "terry towel" type material if possible since this can easily catch toenails.
    b. Avoid squeezing rat too tightly against your body.
b. Ensure animal can breathe and is not over-heating at all times.
    a. Monitor colour of feet
        1. If they are turning blue, the rat is not getting enough oxygen and must immediately be released from the towel.
        2. If they are going bright red, the rat is over-heating and must immediately be released from the towel.
c. Place rat in the middle of the towel with its head facing a corner. Hold the rat around the shoulder and chest to prevent it from walking off the towel (see Figure A).

Figure A

d. Wrap the corner of the towel over the head while still holding the rat around its shoulders and chest (see Figure B).

Figure B

e. Grasp the rat's shoulder and chest through the towel with the hand that folded the towel over the rat's head and pull the other hand out of towel (see Figure C).

Figure C

f. Wrap one side of the towel around the rat's body and tuck it around the rat (see Figures D and E).

Figure D

Figure E

g. Wrap the other side of the towel around the rat's body and tuck it around the rat (see Figure F).

Figure F

h. The wrapped rat can then be held against the body (Figure G), on a flat surface against the body (Figure H) or tucked into a tube (Figure I). Ensure the rat is monitored for colour of extremities to ensure that it can breathe and is not overheating.

Figure G

Figure H

Figure I