University Animal Care Committee Standard Operating Procedure		
Document No: 10.10	**Subject:** Blood Collection in Rats	
Date Issued: July 7, 2011	**Revision:** 4	**Page No:** 1

Location: Queen's University

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

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe the most common methods of blood collection in rats: saphenous vein, tail vein, jugular vein, and cardiac puncture.

1. Introduction:

The following are "good practice" guidelines recommended for blood collection volumes collection sites and needle gauges.

- The Circulating Blood Volume (CBV) of an adult rat is ~64 ml/kg (0.064ml/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 a week.
- 10% (maximum) of the CBV can be collected in a single collection every 2 weeks.
- 15% (maximum) of the CBV can be collected in a single collection every 4 weeks.

To calculate blood collection volumes:

- Body Weight x Circulating Blood Volume = Total Blood Volume (TBV)
- TBV x % blood sample required = acceptable volume to be collected
  (i.e. 100 g x 0.064 ml/g = 6.4 ml/g ***then*** 6.4 x 0.075 = 0.5 ml is the max accepted volume)

Body Weight (g)	Total Circulating Blood Volume (mL/g)	Acceptable volume for collection µl (mL)		
		7.5% Single collection/ 1 week	10% single collection/ 2 weeks	15% single collection/ 4 weeks
100	6.4	500 (0.5 ml)	600 (0.6 ml)	900 (0.9 ml)
150	9.6	700 (0.7)	900 (0.9)	1400 (1.4)
200	12.8	900 (0.9)	1200 (1.2)	1900 (1.9)
250	16	1200 (1.2)	1600 (1.6)	2400 (2.4)
300	19.2	1400 (1.4)	1900 (1.9)	2800 (2.8)
350	22.4	1600 (1.6)	2200 (2.2)	3300 (3.3)
400	25.6	1900 (1.9)	2500 (2.5)	3800 (3.8)
450	28.8	2100 (2.1)	2800 (2.8)	4300 (4.3)
500	32	2400 (2.4)	3200 (3.2)	4800 (4.8)

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 anemia.

- 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 increased respiratory rate at rest with severe anemia.
- Packed cell volume, haemoglobin level, red blood cell and reticulocyte counts should be monitored throughout the series of bleeds using the results from the first sample from each animal as the baseline for the animal.
- If volumes larger than10% are collected, replace volumes by 3-4 times the blood volume collected with warmed (30-39 degrees) isotonic fluids.

2. Materials:

- Heat lamp
- Restrainers as required
- Sterile syringes (1 – 3 ml)
- Sterile needles (multiple sizes ranging from 23-30g)
- Sterile gauze
- Sterile scalpel blades
- EMLA cream*****
- Alcohol swabs
- Petroleum jelly
- Collection tubes
- Anaesthetics as required
- Hair clippers
- Warm water and disinfecting soap
- Warmed 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:

Use the following table to ascertain the most appropriate site for blood collection based on the volume required.

Site	Tail Vein	Saphenous	Cardiac puncture	Jugular
Multiple sampling	Yes	Yes	No	No
Volume	0.05 - 0.1 ml/site	0.1-0.3 ml	1.0-3.0 ml	1.0 ml
Gauge (maximum)	23	25 (23)	23	25 (23)

- Only University Animal Care Committee (UACC) approved blood collection techniques can be performed.
- The least volume required should be collected at all times.
- All collections should be performed by trained and competent individuals.
- The smallest needle size that complements collection location without causing hemolysis should be used.
- Each and every animal requires a new sterile syringe and a new sterile needle/lancet.
- Only three attempts per site should be practiced. If unsuccessful, allow another (trained and competent) person to collect the sample.
- Apply pressure with gauze until hemostasis occurs.

Saphenous Vein

- Each and every animal requires a new sterile syringe and a new sterile needle/lancet.
- .Prepare your sample tubes, and have them readily available.
- If used, apply EMLA cream to the puncture site and wait 15 minutes for it to take effect.
- The saphenous vein lies dorsal then laterally over the tarsal joint, and is immediately visible under the skin.
- Remove the animal from the cage and restrain with isoflurane anesthesia (as per SOP 10.6) or by securely wrapping them in a towel.
- Grasp the dorsal end of the thigh and extend the back leg ensuring the leg is not hyper extended (Figure 1). If extended too far, it can impede blood flow. Confirm the animal can breathe comfortably.
- Remove hair with clippers and swab the site with alcohol.
- Apply petroleum jelly over the vein. This aids in the formation of a large bead of blood.
- Using the appropriate gauge needle, puncture the vein at a 45° angle cranially (bevel up). Inserting needle in the caudal direction may damage the sciatic nerve. Allow a bead of blood to form and collect .All blood loss must be included in calculated volumes.
- Apply pressure to the site with gauze until hemostasis occurs.

- Administer supplemental SC fluids if necessary, dependent on the blood volume collected.
- Return the animal to its cage and monitor for any signs of distress.

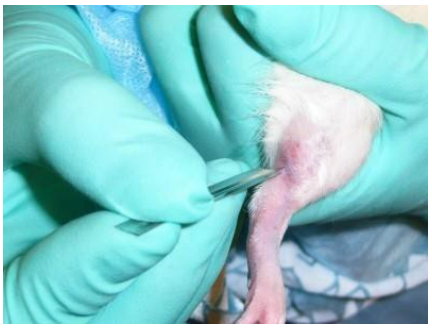

Figure 1

Tail Vein

- Each and every animal requires a new sterile syringe and a new sterile needle/lancet.
- Prepare your sample tubes and have them readily available.
- Release the vacuum on the syringe prior to inserting into vessel.
- Remove the animal from the cage and anesthetize or restrain securely.
- To cause vasodilation, focus the heat lamp on the animal's tail for 5- 10 seconds, or alternatively, warm the animal by placing a heat lamp at a safe distance above the cage for approximately 5 min.
- Ensure the tail is accessible and the animal is comfortable.
- Gently scrub the tail with surgical soap.
- Identify the lateral tail veins. These are used for blood collection.
- If used, apply EMLA cream to the intended site and wait 15min for it to take effect. Hold the distal portion of the tail straight.
- Swab the tail with alcohol. Using the needle, puncture the vein in an upward motion as if the needle is following the path of the vessel.
- Withdraw the needle and collect the drops of blood.
- 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 repeated sampling. Hemostasis must be ensured; a cautery pen may be used post-collection, if needed.
- For the collection of larger volumes, a needle and syringe or a butterfly can be used. Pull back on the plunger slowly so that the vessel doesn't collapse, and obtain desired volume.
- Apply pressure to the site with gauze until hemostasis occurs.
- Return the animal to the cage and monitor for any signs of distress.

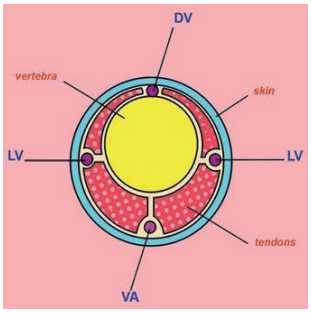

Diagram of a transverse sectional view of a rat tail showing the dorsal vein (DV), lateral veins (LV) and ventral artery (VA). (Modified image reprinted from The Laboratory Rat, G.J. Krinke (Ed.), pp. 491, Copyright 2000)

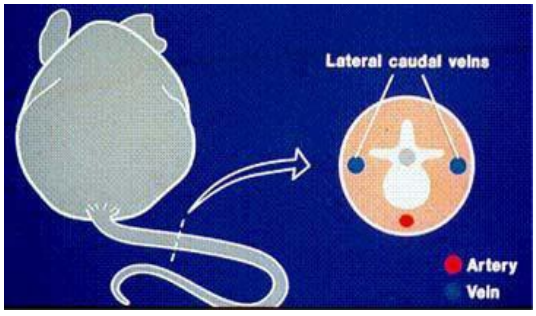

Diagram of a transverse sectional view of mouse tail. Kathryn Flynn, NIH - DVR - SoBran

Jugular Vein

- Each and every animal requires a new sterile syringe and a new sterile needle/lancet.
- Anesthetize the rat following the *SOP 10.6 "Anesthesia in Rats"*.
- Once the animal has reached a surgical plane of anesthesia, place in dorsal recumbency near the edge of the table surface. Angle the head towards the handler, extend the thoracic legs laterally and extend the head in a downward position.
- The jugular runs midway between the shoulder bone and neck just cranial of the collar bone.
- Release the vacuum on the syringe prior to inserting into vessel.
- Palpate for a pulse. If a pulse can't be palpated, the jugular is located midpoint between the sternum and the shoulder. Shave and apply alcohol to the area to allow for better visualization.
- Using a 1 ml syringe and needle, insert the needle 90 degrees to the skin and look for blood in the hub of the needle. Pull back slowly on the plunger to collect the sample.
- Apply pressure to the site with gauze until hemostasis occurs
- Replace lost fluid with 3-4 times the volume collected using isotonic fluids administered subcutaneously.
- Place the animal in sternal recumbency and continue to monitor the animal for signs of distress until it recovers.

Cardiac Puncture

- This is a terminal procedure.
- There are three methods. Dorsal recumbency (lateral and ventral aspirate) and lateral recumbency.
- Anesthetize the rat following the *SOP 10.6 "Anesthesia in Rats"*.
- Once the animal has reached a surgical plane of anaesthesia, position the animal in dorsal or lateral recumbency.

- When sterility is of concern, the skin surrounding the puncture site can be excised to expose the underlying muscle area.
- Release the vacuum on the syringe prior to inserting into vessel.
- Confirm death after exsanguination via a secondary method of euthanasia.

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 a 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.

References:

1) 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)

2) Wolfensohn, S., Lloyd, M. 2nd Edition, Blackwell Science Ltd. 1998.

3) Guidelines for survival bleeding of mice and rats; NIH: http://oacu.od.nih.gov/ARAC/Bleeding.pdf

4) Guide to the Care and Use of Experimental Animals, Vol. 1 (2nd ed), Canadian Council on Animal Care, Canada, 1993: http://ccac.ca/en/CCAC_Programs/Guidelines_Policies/GUIDES/ENGLISH/V1_93/APPEN/APPVIII.HTM

5) The National Centre 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

Revised: January 24, 2012 / March 26, 2012 / September 22, 2015 / February 28, 2019