

University Animal Care Committee Standard Operating Procedure		
Document No: 10.10.1	Subject: Tail Vein Blood Collection in Rats	
Date Issued: July 7, 2011	Revision: 7	Page No: 1

Location: Queen's University

Responsibility: Principal Investigators, Research Staff, Veterinary Staff

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe how to properly collect blood from the tail vein.

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 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)	25	25 (23)	23	25 (23)

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 rat: 42-65 days). The following must be taken into consideration:

- Weight of the animal to be sampled
- Age and health of the animal to be sampled
- Effects of handling stress
- Collection site
- Frequency of sampling necessary
- Training and experience of the personnel performing the collection
- Suitability of sedation and/or anesthesia

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- 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 μ L)*

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 or Lactated Ringer's solution).

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 ~64mL/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. 100g x 0.064mL/g = 6.4mL/g **then** 6.4 x 0.075 = 0.5mL is the max accepted volume)

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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)	600 (0.6)	900 (0.9)
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 than 10% are collected, replace volumes by 3-4 times the blood volume collected with warmed (30-39 degrees) isotonic fluids.

2. Materials:

- Heat source
- Restrainers as required
- Sterile syringes (1 – 3 ml)
- Sterile needles (multiple sizes ranging from 23-30g)
- Sterile gauze

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- Sterile scalpel blades
- Alcohol swabs
- Collection tubes
- Warm water and disinfecting soap
- Warmed isotonic fluids such as Lactated Ringers or 0.9% NaCl
- Topical anesthetic cream (Lidocaine Prilocaine 2.5%)
- Anesthetics as required (if using anesthetics: eye lubricant, heating source, Isotonic fluids – see SOP 10.6 Anesthesia in Rats)
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3. Procedures:

- The minimal 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 animal requires a new sterile syringe and a new sterile needle/lancet.
- If after two attempts there is no success in blood collection, allow another (trained and competent) person to the sample. Only 3 attempts per animal.
- Apply pressure with gauze until hemostasis occurs.

Tail Vein

- Each animal requires a new sterile syringe and a new sterile needle/lancet for each venipuncture.
- 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.
- Using a heat source (heat lamp or seed mat) warm the cage prior to cause vasodilation.
- Ensure the tail is accessible and the animal is comfortable.
- Gently clean the tail with disinfecting soap.
- Identify the lateral tail veins. These are used for blood collection. (*See figure 1 and 2*)
- Apply topical anesthetic 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 bevel up as if the needle is following the path of the vessel.

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- Withdraw the needle and collect the drops of blood.
- For the collection of larger volumes, a needle and syringe can be used. Pull back on the plunger slowly so that the vessel doesn't collapse and obtain desired volume.
- Apply pressure to the collection site with gauze until hemostasis occurs.
- Return the animal to the cage and monitor for any signs of distress.

Figure 1 and 2

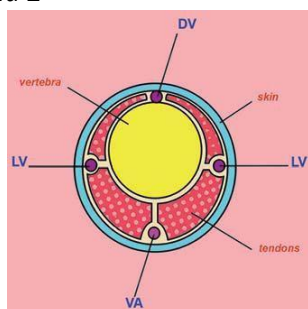


Diagram of a transverse sectional view of a rat tail showing the lateral veins (LV) and ventral artery (VA).
(Modified image reprinted from The Laboratory Rat, G.J Krinke (Ed.), pp. 491, Copyright 2000)

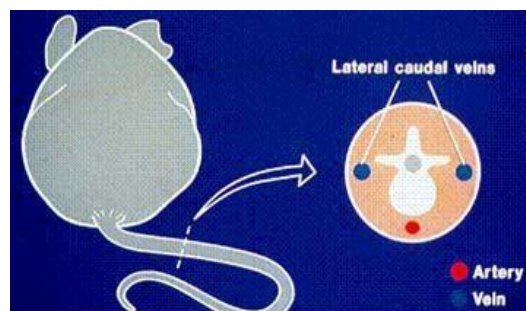


Diagram of a transverse sectional view of mouse tail. dorsal vein (DV), Kathryn Flynn, NIH - DVR - SoBran

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- 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>
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http://ccac.ca/en/CCAC_Programs/Guidelines_Policies/GUIDES/ENGLISH/V1_93/APPEN/APPVIII.HTM
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SOP Revision History:

Date	New Version
January 24, 2012	Triennial review
March 16, 2012	Updated SOP
September 22, 2015	Triennial review
February 28, 2019	Triennial review
February 29, 2022	Triennial review
July 18, 2022	Original SOP separated into different blood collection SOPs
July 20, 2026	Triennial review. Updated wording and needle gauge