

University Animal Care Committee Standard Operating Procedure		
Document No: 10.10.3	Subject: Cardiac Puncture Blood Collection in Rats	
Date Issued: July 7, 2011	Revision: 6	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 via cardiac puncture in a rat.

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)	23	25 (23)	23	25 (23)

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

2. Materials:

- Sterile syringes (1 – 3 ml)
- Sterile needles (multiple sizes ranging from 23-30g)
- Collection tubes
- Anaesthetics

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3. Procedures:

- 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.

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.
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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>

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