

University Animal Care Committee Standard Operating Procedure		
<b>Document No:</b> 10.10.2	<b>Subject:</b> Jugular Vein Blood Collection in Rats	
<b>Date Issued:</b> July 7, 2011	<b>Revision:</b> 6	<b>Page No:</b> 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 jugular 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
<b>Multiple sampling</b>	Yes	Yes	No	No
<b>Volume</b>	0.05 - 0.1 ml/site	0.1-0.3 ml	1.0-3.0 ml	1.0 ml
<b>Gauge (maximum)</b>	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 $\mu$ 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:

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

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- Collection tubes
- Anaesthetics
- Hair clippers
- Warmed isotonic fluids such as Lactated Ringers or 0.9% NaCl

### 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.

#### 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.
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- 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 (2<sup>nd</sup> ed), Canadian Council on Animal Care, Canada, 1993:  
[http://ccac.ca/en/CCAC\\_Programs/Guidelines\\_Policies/GUIDES/ENGLISH/V1\\_93/APPEN/APPVIII.HTM](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