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.

<table>
<thead>
<tr>
<th>Site</th>
<th>Tail Vein</th>
<th>Saphenous</th>
<th>Cardiac puncture</th>
<th>Jugular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple sampling</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Volume</td>
<td>0.05 - 0.1 ml/site</td>
<td>0.1-0.3 ml</td>
<td>1.0-3.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Gauge (maximum)</td>
<td>23</td>
<td>25 (23)</td>
<td>23</td>
<td>25 (23)</td>
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</tbody>
</table>

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)
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 mucus membranes, cold skin and extremities, restlessness, hyperventilation, and a sub-normal body temperature.
- Signs of anemia include pale mucus 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
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.
**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:**


**SOP Revision History:**

<table>
<thead>
<tr>
<th>Date</th>
<th>New Version</th>
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<tbody>
<tr>
<td>January 24, 2012</td>
<td>Tri-annual review</td>
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<tr>
<td>March 16, 2012</td>
<td>Updated SOP</td>
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<tr>
<td>September 22, 2015</td>
<td>Tri-annual review</td>
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<td>February 28, 2019</td>
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<td>February 29, 2022</td>
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<td>July 18, 2022</td>
<td>Original SOP separated into different blood collection SOPs</td>
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