

| University Animal Care Committee Standard Operating Procedure |                                                  |                      |
|---------------------------------------------------------------|--------------------------------------------------|----------------------|
| <b>Document No:</b><br>7.10.5                                 | <b>Subject: Cardiac Blood Collection in Mice</b> |                      |
| <b>Date Issued:</b><br>March 14, 2012                         | <b>Revision:</b><br>6                            | <b>Page No:</b><br>1 |

**Location:** Queen's University

**Responsibility:** Principal Investigators, Research Staff, Veterinary Staff

**Purpose:** The purpose of this Standard Operating Procedure (SOP) is to describe the methods of cardiac blood collection in mice.

### 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 determine the most appropriate site for blood collection based on the volume required.

| Site                     | Submandibular   | Saphenous  | Submental       | Tail Vein      | Retro-orbital  | Cardiac Puncture |
|--------------------------|-----------------|------------|-----------------|----------------|----------------|------------------|
| <b>Multiple sampling</b> | Yes             | Yes        | Yes             | Yes            | Yes            | No               |
| <b>Volume</b>            | Max.200µl       | Max. 200µl | Max. 200µl      | 50µl           | Max. 200µl     | TBV              |
| <b>Gauge Needle</b>      | 4-5.5 mm lancet | 23-25g     | 4-5.5 mm lancet | 23-25g/scalpel | Capillary tube | 23-25g           |

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 mouse: 38-47 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
- 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 µL)*

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

| University Animal Care Committee Standard Operating Procedure |                                                  |                      |
|---------------------------------------------------------------|--------------------------------------------------|----------------------|
| <b>Document No:</b><br>7.10.5                                 | <b>Subject: Cardiac Blood Collection in Mice</b> |                      |
| <b>Date Issued:</b><br>March 14, 2012                         | <b>Revision:</b><br>6                            | <b>Page No:</b><br>2 |

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 Circulating Blood Volume (CBV) of an adult mouse is ~72 ml/kg (0.072 ml/g).

To calculate blood collection volumes:

Total Blood Volume (TBV) = Body weight x Circulating Blood Volume

- 1% (maximum) of the TBV can be collected every 24 hours.
- 7.5% (maximum) of the TBV can be collected in a single collection, once per week.
- 10% (maximum) of the TBV can be collected in a single collection, once per every 2 weeks.
- 15% (maximum) of the TBV can be collected in a single collection, once per every 4 weeks.

Example:

- $TBV \times \% \text{ (based on desired frequency of collection)} = \text{allowable volume to be collected.}$
- **i.e. For a single collection once per week:**  $20 \text{ g} \times 0.072 \text{ ml/g} = 1.44 \text{ ml/g}$  **then**  $1.44 \times 0.075 \text{ (7.5\% for once per week sample)} = 0.1 \text{ ml}$  is the maximum allowable volume.

| Body Weight (g) | Total Circulating Blood Volume (ml) | Acceptable volume for collection $\mu\text{l}$ (ml) |                                      |                                              |                                              |
|-----------------|-------------------------------------|-----------------------------------------------------|--------------------------------------|----------------------------------------------|----------------------------------------------|
|                 |                                     | 1.0% cumulative or single collection every 24 hrs.  | 7.5% single collection once per week | 10% single collection once per every 2 weeks | 15% single collection once per every 4 weeks |
| 15              | 1.08                                | 11 $\mu\text{l}$                                    | 80 (0.08)                            | 108 (0.11)                                   | 160 (0.16)                                   |
| 20              | 1.44                                | 14 $\mu\text{l}$                                    | 108 (0.11)                           | 144 (0.14)                                   | 216 (0.21)                                   |
| 25              | 1.80                                | 18 $\mu\text{l}$                                    | 135 (0.14)                           | 180 (0.18)                                   | 270 (0.27)                                   |
| 30              | 2.16                                | 22 $\mu\text{l}$                                    | 162 (0.16)                           | 216 (0.22)                                   | 300 (0.33)                                   |
| 35              | 2.52                                | 25 $\mu\text{l}$                                    | 189 (0.19)                           | 252 (0.25)                                   | 375 (0.37)                                   |
| 40              | 2.88                                | 29 $\mu\text{l}$                                    | 216 (0.22)                           | 288 (0.29)                                   | 430 (0.43)                                   |

| University Animal Care Committee Standard Operating Procedure |                                                  |                      |
|---------------------------------------------------------------|--------------------------------------------------|----------------------|
| <b>Document No:</b><br>7.10.5                                 | <b>Subject: Cardiac Blood Collection in Mice</b> |                      |
| <b>Date Issued:</b><br>March 14, 2012                         | <b>Revision:</b><br>6                            | <b>Page No:</b><br>3 |

This schedule allows for recovery time for the animals as illustrated in the following table:

| Percent of blood volume collected in a <b>SINGLE</b> sampling | Recovery period (weeks) | Percent of blood volume collected over a 24-HOUR PERIOD ( <b>MULTIPLE</b> samples) | Recovery period (weeks) |
|---------------------------------------------------------------|-------------------------|------------------------------------------------------------------------------------|-------------------------|
| 7.5%                                                          | 1                       | 7.5%                                                                               | 1                       |
| 10%                                                           | 2                       | 10 - 15%                                                                           | 2                       |
| 15%                                                           | 4                       | 20%                                                                                | 4                       |

## 2. Materials:

- Sterile syringes
- Sterile needles (23-25g)
- Gauze
- Alcohol swabs
- Collection tubes
- Anaesthetics
- Surgical tools as needed

## 3. Procedures:

- All collections should be performed by trained and competent individuals.
- Each animal requires a new sterile syringe and a new sterile needle. Prepare all equipment in advance.

### Cardiac Puncture

- Each animal requires a new sterile syringe and a new sterile needle. This is a terminal procedure.
- There are three methods: dorsal recumbency (lateral and ventral aspirate) and lateral recumbency.
- Anesthetize the mouse following the *SOP 7.6 "Anaesthesia in Mice"*.
- When sterility is of concern, the skin surrounding the desired puncture site can be excised to expose the underlying muscle area for needle insertion.
- Once the animal has reached a surgical plane of anaesthesia, lay the animal either in dorsal recumbency or lateral recumbency.
- Release the vacuum on the syringe prior to skin puncture.

| University Animal Care Committee Standard Operating Procedure |                                                  |                      |
|---------------------------------------------------------------|--------------------------------------------------|----------------------|
| <b>Document No:</b><br>7.10.5                                 | <b>Subject: Cardiac Blood Collection in Mice</b> |                      |
| <b>Date Issued:</b><br>March 14, 2012                         | <b>Revision:</b><br>6                            | <b>Page No:</b><br>4 |

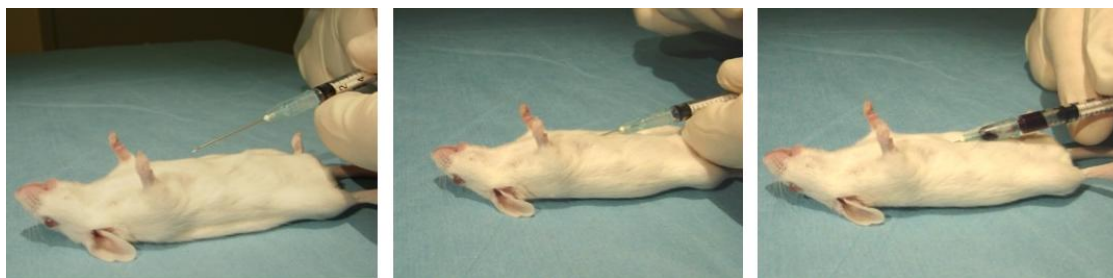
*Dorsal Recumbency (Lateral aspirate)*

- 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 15-20 degree 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 heartbeat is the strongest.
- When there is evidence of blood within the hub of the needle, steady the syringe and withdraw blood slowly.

| University Animal Care Committee Standard Operating Procedure |                                                  |                      |
|---------------------------------------------------------------|--------------------------------------------------|----------------------|
| <b>Document No:</b><br>7.10.5                                 | <b>Subject: Cardiac Blood Collection in Mice</b> |                      |
| <b>Date Issued:</b><br>March 14, 2012                         | <b>Revision:</b><br>6                            | <b>Page No:</b><br>5 |

### References:

- 1) Hoff, Janet, LVT, RLATG. "Methods of Blood Collection in the Mouse", *J. Lab Animal*, Vol 29, No. 010 (November 2000).
- 2) Kathryn Flynn, NIH - DVR – SoBran
- 3) The National Center 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>
- 4) <http://www.usp.br/bioterio/Artigos/Procedimentos%20experimentais/Bleeding.pdf>
- 5) [https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/rodent\\_bleeding.pdf](https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/rodent_bleeding.pdf)
- 6) <https://animal.research.uiowa.edu/iacuc-guidelines-blood-collection> 7)
- Regan, et al. *JAALAS*, 2016. 55 (5):570-576.
- 8) University of Washington Training Manual
- 9) PennState Wet Lab for Teaching Common Rodent Techniques
- 10) 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)
- 11) Guidelines for survival bleeding of mice and rats; NIH: <http://oacu.od.nih.gov/ARAC/Bleeding.pdf>
- 12) Guide to the Care and Use of Experimental Animals, Vol. 1 (2<sup>nd</sup> ed), Canadian Council on Animal Care, Canada, 1993
- 13) Physiological and Pathological Impact of Blood Sampling by Retro-Bulbar Sinus Puncture and Facial Vein Phlebotomy in Laboratory Mice, Teilmann et al., PLOS Published: November 26, 2014  
<https://doi.org/10.1371/journal.pone.0113225>

### SOP Revision History:

| Date               | New Version                                                  |
|--------------------|--------------------------------------------------------------|
| September 22, 2015 | Triennial review                                             |
| January 25, 2018   | Triennial review                                             |
| February 28, 2019  | Updated SOP                                                  |
| February 28, 2022  | Triennial review                                             |
| July 18, 2022      | Original SOP separated into different blood collections SOPs |
| July 20, 2026      | Triennial review. Wording updated                            |
|                    |                                                              |
|                    |                                                              |