

Research Animal Standard Operating Procedures (SOP) must meet the following criteria:

1. Describe procedures or activities involving research animal(s) common to more than one research project.
2. Support the handling and or performance or undertaking of a procedure(s), involving an animal, in the same way on each occasion it is performed.
3. Describe a procedure or activity involving a research animal(s) undertaken by more than one person; and
4. Describe a procedure or activity involving a research animal(s) that will be undertaken in more than one location.

Name of Procedure	Blood collection – Saphenous vein	
Species	Mouse	
ACEC	Reference	SOP#73 – Jul 2023 - Blood collection, saphenous vein- mouse
	Author	Jenny Smart
	Version	1.4
	Date approved	28 July 2023
	Date for review	28 July 2026
	Procedure classification	3
Ethical considerations	<ol style="list-style-type: none"> 1. Respect for animals must underpin all decisions and actions involving the care and use of animals for scientific purposes. 2. The procedure must be performed according to current best practice to support the wellbeing of the animal. 3. Persons performing this procedure must be competent in the procedure or be under the direct supervision of someone who is competent. 	

Details

1. Site of Blood Collection

Lateral Saphenous vein

2. Description of procedure, including restraint and collection methods

The method described in this SOP follows that set out by Norecopa, Norway's National Concensus Platform for the advancement of the 3Rs. Photographs are courtesy of that site and an instructional video of the procedure is available from the site (see reference 1.)

1. MATERIALS

- 1.1 Sterile hypodermic needle - 23-25G
- 1.2 Blood collection device (e.g. microhaematocrit capillary tube)
- 1.3 Scalpel blade - size 10 or 15
- 1.4 Antiseptic (e.g. hibiclens, microshield- chlorhexidine based surgical scrub)
- 1.5 Vaseline
- 1.6 Restraint device - mouse bag, falcon tube with air holes or similar
- 1.7 Heat lamp (optional)
- 1.8 Weighing device

2. PROCEDURES

- 2.1 Set up work surface with the above materials.
- 2.2 Weigh mouse and calculate approximate blood volume and maximum blood collection volumes based on an average of 70-80 mls blood/ kg weight and maximum volumes as shown in section 3.
- 2.3 Place the mouse in a restraint device such as a falcon tube with air holes or 'mouse bag'. Extract the tail and one hind limb from the restraint.
- 2.4 The lateral saphenous vein is located on the lateral surface of the hind limb between the hock and the stifle.
- 2.5 Wet the hair between the hock and stifle with the soapy water and use the scalpel blade to carefully shave the hair from over the vein.



Saphenous vein



- 2.6 Spread a small amount of Vaseline over the skin overlying the vein. This will cause the blood to bead rather than spread, making it easier to collect.
- 2.7 Collection:
 - (i) Restrain the hind limb of the mouse with the thumb and forefinger so that the skin is taut above the vein. This will cause the vein to dilate.
 - (ii) Prick the vein with the point of the needle. Blood should immediately begin to bead on the Vaseline treated skin.
 - (iii) Collect the blood into a microhaematocrit tube or allow it to drip into a

- blood collection tube. Collect no more blood than the maximum volumes described in the table below.
- (iv) Once the blood sample is collected, apply pressure to the venipuncture site with a dry cotton swab to encourage haemostasis.
 - (v) Gently wash away any traces of blood before returning the mouse to its cage.



NOTE: Discard all garbage into appropriate containers and clean the work area.

3. Maximum volume of blood will be collected?

Single Bleed - maximum removable volume will be no more than 10% of the animal's blood volume (see table below).

Multiple samplings - maximum removable volume on a daily basis will be no more than 1% of the animal's blood volume (see table below).

Mouse		
Body Weight	20 gms	30 gms
Estimated whole blood volume (mls/kg)	70-80	70-80
Blood volume (mls)	1.4- 1.6	2.1- 2.4
Volume to be removed – single collection (10% of blood volume)	0.14- 0.16 mls	0.21- 0.24 mls
Volume to be removed – Multiple collections (1% of blood volume/day)	0.014-0.016 mls	0.021- 0.024 mls

4. How will the animal be monitored for the effects of acute blood loss?

Signs to be monitored as indicative of acute blood loss in the mouse include pale ears and feet, cold skin and extremities, restlessness, hyperventilation, and a subnormal body temperature.

Where multiple blood collections are to be performed:

5. Detail the total number of blood collections, and the time interval between each collection

Repeated blood samples of 10% of total blood volume can be collected at interval of two- to three- week intervals or 1% of total blood volume on a daily basis. In these instances, the animal must be monitored for effects of chronic blood loss (see Section 6).

6. How will the animal be monitored for the effects of chronic blood loss?

Signs to be monitored as indicative of anaemia from chronic blood loss include:

- pale mucous membranes (conjunctiva or inside the mouth)
- pale tongue, gums, ears or footpads (if non-pigmented)
- increased respiratory rate when at rest (at the extreme level).

Additional monitoring should be performed when frequent blood collections are to be performed, with monitoring of the individual animal using its own baseline established at beginning of collection period. Monitoring parameters should include:

- packed cell volume
- haemoglobin level
- red cell count
- reticulocyte count

Peripheral blood smears can be examined in order to detect early changes associated with anaemia, for example, polychromasia of the red cells.

References

1. Norecopa – Norwegian National Consensus Platform for the advancement of the 3Rs
<https://norecopa.no/education-training/films-and-slide-shows>

ACEC Chair

