

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	Rat	
ACEC	Reference	SOP#121-Mar22-Blood collection- saphenous vein- rat
	Author	Jenny Smart
	Version	1.1
	Date approved	25 February 2022
	Date for review	24 February 2025
	Procedure classification 1. Observation involving minor interference 2. Animal unconscious without recovery 3. Minor conscious intervention 4. Minor surgery with recovery 5. Major surgery with recovery 6. Minor physiological challenge 7. Major physiological challenge	3
Ethical considerations	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

Site of Blood Collection

Lateral Saphenous vein

Description of procedure

Description of procedure, including restraint methods and collection methods.

1. MATERIALS

- 1.1 Sterile hypodermic needle- 23G
- 1.2 Blood collection device (e.g. microhaematocrit capillary tube eppendorf or test tube)
- 1.3 Electric clippers
- 1.4 Antiseptic solution (eg chlorhexidine)
- 1.5 Vaseline
- 1.6 Restraint device- small black cloth bag
- 1.7 Heat lamp (optional)
- 1.8 Weighing device

2. PROCEDURES

- 2.1 Set up work surface with the above materials.
- 2.2 Weigh rat and calculate approximate blood volume and maximum blood collection volumes based on an average of 70 mls blood/ kg weight and maximum volumes as shown in section 3.
- 2.3 Place the rat in a restraint device such as a 'rat bag' a dark cloth bag and extract 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 Aseptic technique should be used: hair around the collection site should be removed and the skin aseptically prepared e.g. with a chlorhexidine solution.
- 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.



- 3. COLLECTION**
- (i) Restrain the hind limb of the rat pressing fingers over the saphenous vein just proximal to the site of blood collection, 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.
 - (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 rat to its cage.
 - (vi)
- NOTE:** Discard all garbage into appropriate containers and clean the work area.

Maximum volume of blood to 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).

	Rat	
Body Weight	200g	400g
Estimated whole blood volume (mls/kg)	70	70
Blood volume (mls)	14	28
Max Volume to be removed – single collection (10% of blood volume)	1.4	2.8
Max Volume to be removed – Multiple collections (1% of blood volume/day)	0.14	0.28

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 rat include pale ears and feet, cold skin and extremities, restlessness, hyperventilation, and a subnormal body temperature.

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.

Where multiple blood collections are to be performed:

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 an interval of two weeks 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 above).

References

1. National Centre for the Replacement Refinement and Reduction of animals in Research – saphenous vein blood sampling in the rat
<https://www.nc3rs.org.uk/rat-saphenous-vein-non-surgical>
2. Norwegian reference centre for laboratory animal science and alternatives.
<https://norecopa.no/films-and-slide-shows/rat#/42550>

ACEC Chair

