Flinders University

Safe Work Method Statement

Rat – Blood Collection 18/06/19

College of Medicine and Public Health Animal Facility

SWMS Number	RA Number	RA Score	
SWMS- 2.2	RA- 2.2	MEDIUM	
Contact Person	**SWMS prepared by**	**AWC Approval Date**	**Review Date**
Roxanne Collingwood	Roxanne Collingwood and Lewis Vaughan	18/06/2019	June 2021

Contents

The SWMS **Rat – Blood Collection** contains the following sections:

- Legislation
  - University Policy
  - Local Policy
  - Safe Work Method Statement
  - Personal Protective Equipment Required
  - Hazards and Controls
  - Before Work Commences
  - General Information
- NHMRC Blood Collection Guidelines
- Saphenous Vein Blood Collection
- Tail Vein Blood Collection Method
- Cardiac Blood Collection – Terminal
- Abdominal Aorta Blood Collection – Terminal

Legislation

- *Australian Code for the Care and Use of Animals for Scientific Purposes 8th Ed.*
- *Animal Welfare Act 1985*
- *Animal Welfare Regulations 2012*
- *Gene Technology Act 2000* (the Act)
- *Gene Technology Regulations 2001*
- *Work Health and Safety Regulations 2012*

University Policy

- Work Health and Safety Policy 2013
- Responsible Conduct of Research Policy 2016
- NHMRC Guidelines

Local Policy

Use of the College of Medicine and Public Health Animal Facilities by all staff and researchers of the College of Medicine and Public Health, Flinders University, is subject to awareness of, and adherence to the following:

Research Involving Animals:

- The University holds a licence for the use of animals for teaching and research purposes. To satisfy the requirements of the licence, anyone wishing to undertake teaching and research using animals must submit a proposal to the Animal Welfare Committee (via the Animal Ethics Review Sub- Committee. No work with animals may commence until written approval has been received from the Animal Welfare Committee. Standardised application forms for Research and Teaching can be found on the Flinders University website listed below. It is your responsibility to regularly check this site for updates to guidelines, forms etc
  http://www.flinders.edu.au/research/researcher-support/ebi/animal-ethics/animal-ethics_home.cfm

---

- **All staff and students involved in animal research must complete Animal Ethics Online Training (AEOT) and must also regularly attend Animal Researcher Information Sessions (ARIS).**

Safe Work Method Statement

Refer to Risk assessments, Safe Work Method Statement for chemicals, processes and plant equipment where appropriate. All projects must have an accompanying Risk Assessment signed by the Animal Facility Manager

SWMS 2.0 Rat- Sexing, Handling, Restraint and Ear Notching
RA 2.0 Rat- Sexing, Handling, Restraint and Ear Notching
SWMS 2.1 Rat- Injection techniques
RA 2.1 Rat- Injection techniques
SWMS 7.0 Compliance - Emergency Contingency
RA 7.0 Compliance - Emergency Contingency
SWMS 7.1Compliance -Transportation
RA 7.1 Compliance -Transportation
SWMS 7.2 - Rodent Importation
RA 7.2- Rodent Importation

Personal Protective Equipment Required

- **Gloves**
- **Gown**
- **Mask**
- **Hair Net**
- **Shoe Covers**

Hazards and Controls

- **Animal bites- training, demonstrate competency, adhere to SWMS.**
- **Animal Scratches- training, demonstrate competency, adhere to SWMS.**
- **Animal Allergies- wear PPE when handling or handling dirty cages to stop the potential development.**
- **Needle Stick- DO NOT recap needles, dispose immediately into sharps containers, adhere to SWMS.**
- **Chemical exposure- wear PPE and goggles.**

Before Work Commences

Ensure that you are aware of the locations of the following:

- **Spill Kit**
- **Fire Extinguisher**
- **Eye Wash**
- **Exits**

Risk Assessment and SDS (Safety Data Sheet) - Ensure that you have read and understood for all the substances being used.

Equipment

- **Check for safety and electrical compliance**
- **Ensure that you have read and understood the Risk Assessment and Safe Work Method Statement**
- **Obtain training before using any equipment**

General Information

- **All procedures are to be performed by trained competent staff.**
- **Training is available from senior animal house staff or Animal Welfare Officer.**
- **Evidence of training is available in the "Training Needs Analysis"**

NHMRC Blood Collection Guidelines

- The approximate blood volume can be calculated using the assumption that the animals total blood volume is 7% of animal's bodyweight. Blood volume limits can be expressed as a percentage of blood volume, assuming that an animal's total blood volume can be estimated at 70mL/kg.
- Up to 10% of the circulating blood volume can be taken on a single occasion from normal healthy animals with minimal adverse effect. This volume may be repeated after 2 weeks.
- For repeat bleeds at shorter intervals, a maximum of 1.0% of an animal's circulating blood volume can be removed every 24 hours.
- Major bleeds require specific AWC project approval. Animals must be given prophylactic fluid therapy in the form of body-temperature Normal Saline for injection by subcutaneous and intraperitoneal injection 15 minutes prior to the procedure. Such animals must be placed in a 30 degree environment for minimum of 30 minutes after the procedure.

- If blood is to be collected into a syringe, a dead space of 0.05 mLs in the luer-nozzle should be considered in the determination of blood sample volumes.

Maximum volumes and recovery periods for blood collection

Period of collection	% of blood volume collected	Approximate recovery period in weeks
Single bleed	Up to 7% (minor bleed)	1
	10% (moderate bleed)	2
	15% (severe bleed)	3
Over a 24-hour period	Up to 7%	1-2
	10%	2-3
	15%	4-6

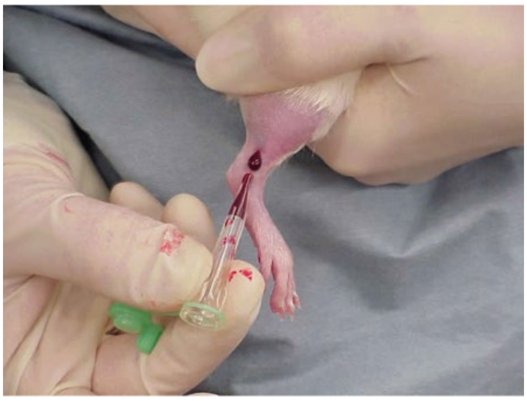
Recommended site and volume of blood collection using calculated blood volume

Species	Total blood volume (using vol. of 7% body weight[mL])	Recommended site for blood collection	<7% Minor bleed (mL)	10% Moderate bleed (mL)	15% Major bleed (mL)
Rat (250gm)	17.5 (0.25g x 70mL/kg)	Saphenous vein, tail vein	<1.30	1.74	2.63

Saphenous Vein Blood Collection

- Anaesthesia or sedation is recommended for Saphenous Vein Puncture, although it is possible to perform the bleed with simple restraint.

1. Restrain the rat with the hind leg extended. Restraining the rat in a light hand towel may assist to calm the animal and improve handling. The lateral saphenous vein is in the hind leg, which runs dorsally and then laterally over the tarsal joint.
2. Immobilise the leg in the extended position by gently applying downward pressure above the knee joint. This technique stretches the skin over the ankle, making it easier to shave, and immobilising the saphenous vein.
3. Carefully shave both lateral and dorsal areas around the ankle and swab with 70% alcohol. The vein should be clearly visible under the skin.
4. A small amount of ‘Vaseline’ may be applied over the site before puncturing the vein; this aids the blood to run freely from the skin and provides more efficient blood collection.
5. A rubber band tourniquet may be applied to the thigh to occlude the vein to improve blood withdrawal.
6. Using a 23 to 25G needle, puncture the vein and use a micro haematocrit tube or microvette to collect the blood as it flows from the puncture site.
7. Once an adequate blood sample has been collected, gentle apply pressure over the wound site to stop further bleeding.
8. Serial sampling may be performed from the same puncture site by gently rubbing off the scab that forms.



Saphenous vein puncture

Tail vein Puncture

Tail Vein Blood Collection Method

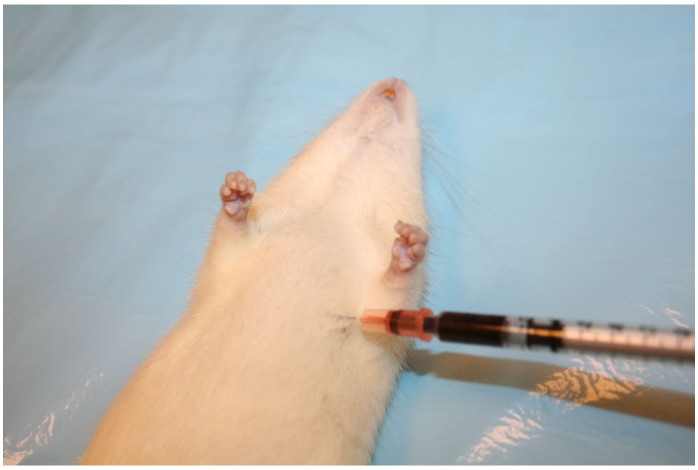
- Anesthesia is not required. Put the rat into a suitably sized restrainer or wrap in a light hand towel and secure.
- The tail vein can be dilated by one of several ways:
  - (i) Occluding the vessel with the thumb and forefinger.
  - (ii) Heating the tail by immersing in warm water (37°C) for 1-2 minutes.
  - (iii) Placing the rat and/or tail under a heat lamp for 3-5 minutes.
  - (iv) Placing the rat in a pre-warmed incubator, set at 35 degrees, for 5 to 10 minutes.
- The lateral (side) veins are immediately below the skin and must be entered at a very shallow angle, almost parallel to the vein. The tail should be bent down while the vein is being entered at the point of the bend.
- Finger pressure or a rubber band may be applied to the base of the tail to act as a tourniquet.
- Using a 21 to 27g needle with the needle bevel up, insert the needle into the vein then release the tourniquet.
- The blood is collected directly into a syringe or a capillary tube (*See picture above*).
- Once an adequate blood sample has been collected, gentle apply pressure over the wound site to stop further bleeding. Further bleeding control may be obtained by using a chemical cautery pen or a small amount of chemical cauteriser applied to the venepuncture site using a cotton bud.

Cardiac Blood Collection - Terminal

1. Animals must be anaesthetised when using this technique. See Rat Anaesthesia and Analgesia Safe Work Method Statement.
2. Position the rat on its back, feel for the heart beat by palpating the mid ventral thorax with your index finger and thumb.
3. Using a 21- 23G needle, carefully insert the needle either between the ribs or beneath the sternum, into the heart. If inserting the needle beneath the sternum, the

needle must be inserted slightly left of the midline, and the needle and syringe should be elevated 10º-20º from the horizontal axis of the sternum.

4. The required volume of blood can then be withdrawn. The animal must be euthanized at the end of the procedure.
5. Death after exsanguination should be ensured with cervical dislocation or exposure to a 100% carbon dioxide atmosphere whilst still under anaesthesia.



Abdominal Aorta Blood Collection - Terminal

- This technique can be used to obtain large quantities of blood of up to 10 to 15 mLs when damage to the heart is contraindicated.
- A general anaesthetic is administered to provide deep surgical anaesthesia.
- A mid-line laparotomy is performed and the abdominal viscera is retracted to expose the abdominal aorta. Removal of connective tissue and finger pressure may be necessary to dilate the blood vessel.
- A 19 to 21 gauge needle attached to a 10 mL syringe or vacutainer is used to penetrate the abdominal aorta and blood is slowly withdrawn.
- The rat should be maintained in deep surgical anaesthesia for the entire procedure and euthanised by severance of the aorta or cervical dislocation at the end of the procedure.

SWMS Review

This SWMS currently applies to the animals housed in the College of Medicine and Public Health Animal Facility. This SWMS will be reviewed 3 yearly, but also updated more frequently as policies, techniques and animal care requirements change.

Position	Name	Contact Details
Manager Animal Facility	Roxanne Collingwood	8204 4380 roxanne.collingwood@flinders.edu.au
Animal Welfare Officer	Lewis Vaughan	0450 424 143 awo@flinders.edu.au

Useful References

https://www.nc3rs.org.uk/rat

http://www.nhmrc.gov.au

http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/home-1

http://www.adelaide.edu.au/ANZCCART/

http://www.flinders.edu.au/research/researcher-support/ebi/animal-ethics/animal-ethics_home.cfm

http://www.medipoint.com/html/for_use_on_mice.html

Any questions regarding the above guidelines and any technical advice/ assistance required can be directed to Animal Facility Manager.