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|---|---|---|
| | **Flinders University**<br>**Safe Work Method Statement**<br>**Mouse – Blood Collection 18/06/19** |  |
| |  | **College of Medicine and Public Health Animal Facility** |

| SWMS Number | RA Number | RA Score | |
|---|---|---|---|
| SWMS- 1.2 | RA- 1.2 | MEDIUM | |
| **Contact Person** | **SWMS prepared by** | **AWC Approval Date** | **Review Date** |
| Roxanne Collingwood | Roxanne Collingwood & Lewis Vaughan | 18/06/2019 | June 2021 |

## Contents

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

## 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).**

- **All personnel working with Genetically Modified Animals (GMO) or working with in a PC1 or PC2 facility must attended a Biosafety Training Day every 3 years**

## Safe Work Method Statement

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

SWMS 1.0 Mouse- Sexing, Handling, Restraint and Ear Notching
RA 1.0 Mouse- Sexing, Handling, Restraint and Ear Notching
SWMS 1.1 Mouse- Injection techniques
RA 1.1 Mouse- Injection techniques
SWMS 1.7 Mouse Transportation
RA 1.7 Mouse Transportation
SWMS 10.2 - Emergency Contingency
RA 10.2 - Emergency Contingency

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

## 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.**
- **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 "Staff Training Needs Analysis".**
- **If sedation or anaesthesia is required for the blood collection procedure SWMS 1.3 *Mouse Anaesthesia and Analgesia* should be followed. Suitable sedatives may include acetylpromazine with or without butorphanol/buprenorphine, or xylazine, or medetomidine for all blood collection in this SWMS, except for cardiac bleeding. General anaesthesia is required for cardiac bleeding. Isoflurane or ketamine/xylazine, ketamine/medetomidine, Zoletil/xylazine or Zoletil/medetomidine, or pentobarbitone may be used for this purpose.**

## 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.

**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 |

**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)** |
|---|---|---|---|---|---|
| Mouse (26gm) | 1.82 (0.026g x 70mL/kg) | Saphenous vein, facial vein, tail vein | <0.12 | 0.18 | 0.27 |

## Saphenous Vein Puncture

- Anaesthesia or sedation is recommended for saphenous vein puncture, although it is possible to perform the bleed with simple restraint.

1. Restrain the mouse in an open falcon tube, with the hind leg extended. 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 the lateral area proximal to the ankle. The vein should be clearly visible under the skin.
4. Apply a small amount of Vaseline over the site before puncturing the vein (this aids the blood to run freely from the skin, and provides more efficient blood collection).

5. Using a 23 to 25g needle, puncture the vein. Use a micro haematocrit tube or microvette tube to collect the blood as it flows from the puncture site.
6. Once an adequate blood sample has been collected, gentle apply pressure over the wound site to stop further bleeding.
7. Serial sampling may be performed from the same puncture site by gently rubbing off the scab that forms.

**Saphenous Vein Puncture**

## Tail Vein Puncture Method

1. The tail vein dilation can be promoted by one of several ways:
    - Occluding the vessel with the thumb and forefinger, or tourniquet.
    - Heat the tail by either immersing in warm water (37°C) for 1-2 minutes.
    - Place the tail of the mouse under a 40 watt incandescent light for up to 5 minutes.
    - Place the mouse in a therma-cage (incubator) set at 35°C for up to 10 minutes.
    - Administering a subcutaneous or intraperitoneal injection of sterile Normal Saline for injection at a dose volume of up to 5% of bodyweight.
    - Administering a subcutaneous or intraperitoneal injection of acetyl-promazine at a dose rate of up to 2.5 mg/kg 15 minutes prior to the blood collection.
2. Smear topical local anaesthetic cream (EMLA Cream) over the surface of the tail 10 minutes prior to the bleeding procedure.
3. General anesthesia is not required, place the mouse into an appropriate size restrainer.
4. The lateral (side) veins are immediately below the skin, and should be entered at a shallow angle to the vein.

5. If using:
    - A scalpel blade, sizes 10, 11, 14, 15, 16, 20, 21 or 22, or a Golden Rod Lancet, and make a light transverse incision with the base of the blade over the tail vein, just sufficient to penetrate the skin and one side of the wall of the vein. See https://www.nc3rs.org.uk/mouse-tail-vein-non-surgical for a video that depicts this technique.

**Tail Vein Puncture**


Available: https://www.nc3rs.org.uk/mouse-tail-vein-non-surgical, accessed: June 29, 2016


Available: https://www.nc3rs.org.uk/mouse-tail-vein-non-surgical, accessed: June 29, 2016

- A 23 to 25 gauge needle, insert the needle into the vein with the bevel upwards. Remove the needle and a small blood drop should form.

6. The blood is collected into a capillary tube or microvette tube, or allowed to drip into a suitable container.
7. Once an adequate blood sample has been collected, gently apply pressure over the wound site to stop further bleeding, or use a chemical cautery pen or a cotton bud impregnated with ferric chloride crystals, sliver nitrate, or potassium permanganate.

## Facial Vein (Cheek Bleed)

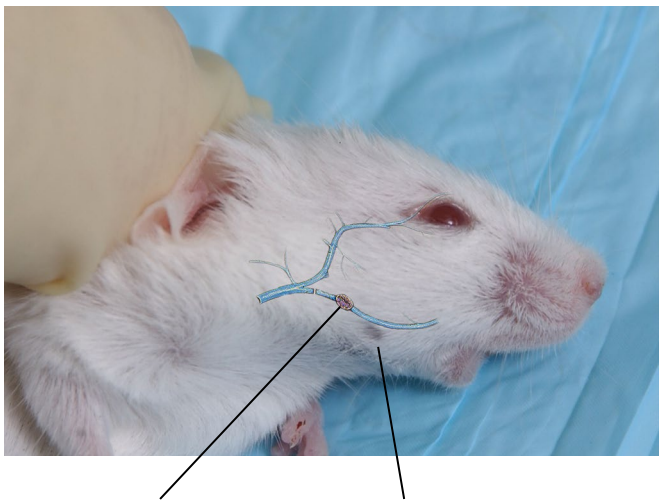
- Anesthesia is not required.
- Note the maximum blood volumes that may be safely collected from a 26 gram mouse:
  - Minor bleeds up to 0.12 mLs – maximum of once weekly.
  - Moderate bleeds up to 0.18 mLs – maximum of once fortnightly.
  - Major bleed up to 0.27 mLs – maximum of once every 3 weeks.
- If a major bleed is collected, the animal should be given 1.0 to 1.5 mLs of body-temperature sterile Normal Saline for Injection 15 minutes prior to the procedure by subcutaneous or intraperitoneal injection. Recovery cages should be placed on warm mats, and supplementary 100% oxygen must be available if signs of shock occur.

1. Select an Eppendorf tube, and fill this with the target volume that has been identified. This will assist you to determine if your collection volumes are too great and whether you need to provide fluid therapy, warmth, and supplementary oxygen.
2. Scruff the mouse (this will cause the eyes to bulge and reduce the flow from the face). Note: Scruff more skin if the mouse is able to move during the procedure. If the mouse's nose and lips start to turn blue, release the mouse and re-scruff using less skin and pressure.
3. Golden rod lancets (*see below for sizes*) should be used.

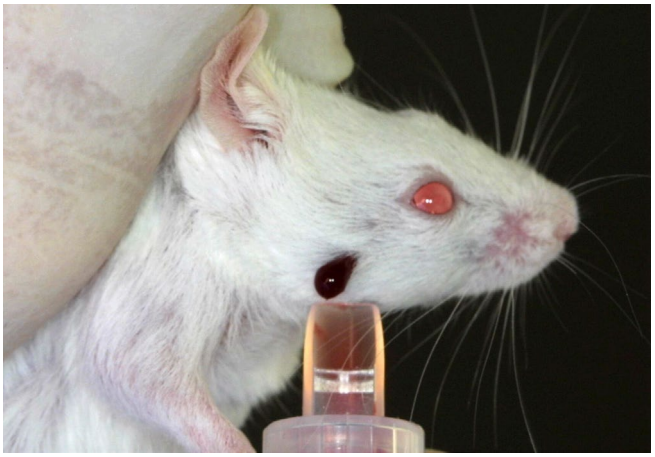
   Lancet sizes - 4 mm point for mice of between 2-8 weeks old.

   - 5 mm point for mice of between 2-6 months of age.

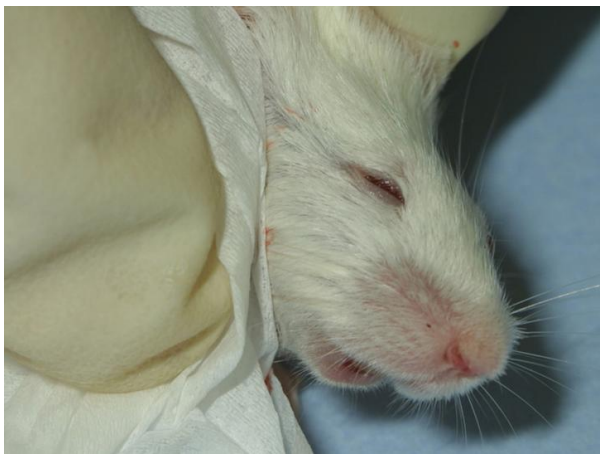
   - 5.5 mm point for mice older than 6 months of age.
4. Insert the needle, or the lancet, one eye length caudal, and one eye width dorsal, to the freckle on the face, and puncture the skin angling the lancet/needle up towards the mouse's ear. The sub-mandibular or facial vein lies just under the skin.
5. Remove the lancet and collect the required volume of blood. If a larger blood sample is required, use your index finger to move the mouse's head gently up and down.
6. Apply gentle pressure until bleeding ceases.



**Submandibular Vein** **Freckle**



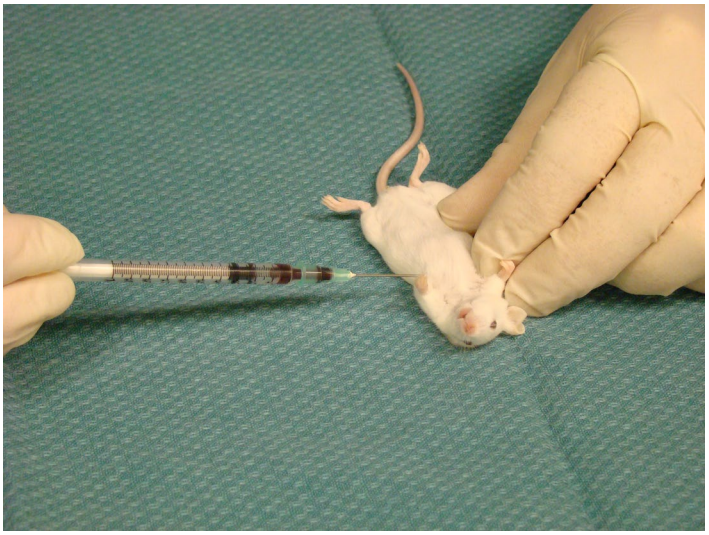
**Blood Collection into tube**



**Apply gentle pressure until bleeding stops**

## Cardiac Puncture - Terminal

1. Animals must be surgically anaesthetised when using this technique. *See "Mouse Anaesthesia Safe Work Method Statement".*
2. Position the mouse on its back, and feel for the heart beat by palpating the mid ventral thorax with your index finger and thumb.
3. Using a 23 to 25G 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. Alternative approaches for cardiac blood collection, which may be used are sternal and diaphragmatic.
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 confirmed by cervical fracture whilst still under anaesthesia.



## 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<br>roxanne.collingwood@flinders.edu.au |
| Animal Welfare Officer | Lewis Vaughan | 0450 424 143<br>awo@flinders.edu.au |

**Useful References**

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.