Flinders University

Safe Work Method Statement

Rat – Injection Techniques

17/09/19

College of Medicine and Public Health Animal Facility

SWMS Number	RA Number	RA Score	
SWMS- 2.1	RA- 2.1	MEDIUM	
Contact Person	**SWMS prepared by**	**AWC Approval Date**	**Review Date**
Lewis Vaughan/Roxanne	Lewis Vaughan/Roxanne Collingwood	17/09/2019	September 2022

Contents

The SWMS **Rat – Injection Techniques** contains the following sections:

Legislation

- *Animal Welfare Act 1985*, South Australian Government
- *Animal Welfare Regulations 2012*, South Australian Government
- *Gene Technology Act 2000* (the Act), Government of Australia
- *Gene Technology Regulations 2001*, Government of Australia.
- *Work Health and Safety Regulations 2012*, South Australian Government

Codes and Policies

- Work Health and Safety Policy 2017
- NHMRC, 2013, *Australian Code for the Care and Use of Animals for Scientific Purposes,* 8th Ed, Australian Government.
- NHMRC, 2018, *The Australian Code for the Responsible Conduct of Research*, Australian Government.
- NHMRC, 2008, *Guidelines to promote the wellbeing of animals used for scientific purposes*, Australian Government.

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. 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), regularly attend Animal Researcher Information Sessions (ARIS) and must be supervised or be competent in the procedures they perform with animals.**

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
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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 animals or handling dirty cages to stop the potential development**
- **Needle Stick- DO NOT recap needles, use single-use 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 Statements**
- **Obtain training under supervision or competency assessment before using any equipment**

General Information

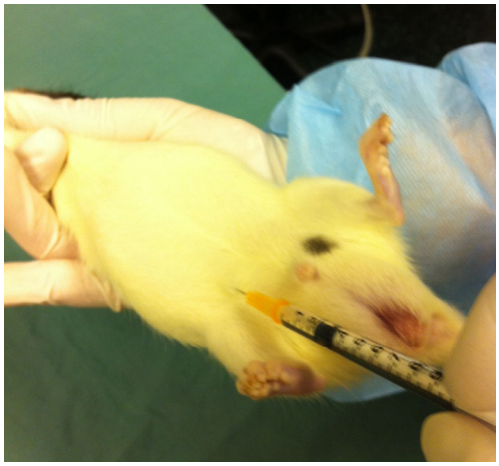
- **All procedures are to be performed by competent personnel.**
- **Training is available from the AWO or appropriate nominated/acting veterinarian or senior animal house staff.**
- **Evidence of competency is recorded in the "Competency Skills Register"**

Intra-Peritoneal Injection

- Needle size 23 – 26G x 13-25mm. The NHMRC (2008) recommended maximum bolus volume is 1% of animal's body weight. If a larger volume is required seek the advice of the AWO or appropriate nominated/acting veterinarian.

1. Using a syringe and a 23 - 26 gauge needle, draw up the required dose, expel any air bubbles. Short, 12 mm, 26 gauge, needles are preferred for rat intra-peritoneal injections. Needle size, however, also depends on the size of the rat and the agent being injected. Consult the AWO or appropriate nominated/acting veterinarian for selecting the correct needle size combination.
2. Gently tip rat downwards with its nose pointing towards the floor, at 45 degrees to the horizontal, this allows the internal abdominal organs to move in a cranial direction. Alternative holding procedures may be used in consultation with the AWO or appropriate nominated/acting veterinarian.

3. Insert the needle, at a 30 to 45 degree angle, into the caudal right quadrant of the abdomen, as shown (*See Photo: Intra-peritoneal injection*).
4. Gently draw back on the plunger, no fluid should be drawn up into the hub of the needle, then inject the required dose.



Intra-peritoneal injection

subcutaneous injection

Subcutaneous Injection

- Needle size 23 – 26G x 13 - 25mm. The NHMRC (2008) recommended single injection volume range is 2 to 5 mL. If a larger volume is required seek the advice of the AWO or appropriate nominated/acting veterinarian. Smaller needle sizes are preferred depending on the size of the rat and the agent being administered.
- Anaesthesia is not usually required. This technique usually requires only one person.

1. Using a 1 to 3 ml syringe and a suitable gauge needle, draw up the required dose, and expel any air bubbles.
2. The injections are usually made under the skin of the back and flank, or under the skin overlying the neck. Avoid the placing injections on the sides of the chest.
3. Place the animal on a table and restrain. An alternative method of restraint is to partially wrap the rat in a light towel or drape.
4. Tent the skin over the shoulders between your thumb and forefinger.
5. Insert the needle through the skin in a cranial direction (*as shown above*) at a shallow angle, 30 degrees, to the skin surface.
6. Drawing back on the plunger may be used to confirm needle placement. If blood is not observed in the hub of the needle change the site of the injection.
7. Slowly withdraw the needle.

Intradermal Injection

- Needle size 27 – 30G x 13mm, maximum number of sites is 6, maximum volume is 0.05ml/site, this, depends on the thickness of the skin and the agent being injected. Seek the advice of the AWO or appropriate nominated/acting veterinarian if a variation to this recommendation is required.
- This technique usually requires two people, one to restrain the animal the other to carry out the injection. Light anaesthesia may be used if one person is administering the injection.
- Sites commonly used include the skin over the back and ventral abdomen, or over the palmar/plantar surface of one hind foot.

1. Shave the area to be injected.
2. With the bevel of the needle up, insert the needle almost parallel with the surface of the skin.
3. Insert the needle into the skin approximately 2-3mm, inject desired dose. Note: There may be considerable resistance to the passage of the needle when it is being inserted into the dermis compared to a subcutaneous injection.
4. A bleb in the skin (*as seen in the photo below*) will indicate a successful intradermal injection. Leave the needle in the injection site for at least one second after injection and slowly withdraw the needle.

Intradermal Injection

Intramuscular Injection

- Needle size 25 – 30G, less than 13mm, maximum volume 0.1ml per site. Note that this is a potentially painful procedure for the animal. Small volumes, small needles and slow injection rate will reduce the pain experienced by the animal.
- This technique requires two people if anaesthesia is not used. Anaesthesia may be used to assist in restraint and the reduction of pain experienced by the animal.
- Intramuscular injections are delivered into the muscles of the hind limb, either the biceps femoris, or the quadriceps.

1. Restrain the rat and extend the hind limb. Palpate the muscle and femur with the fingertips.
2. Insert the needle cranial or caudal to the femur halfway between the hip and the knee, and parallel to the femur.

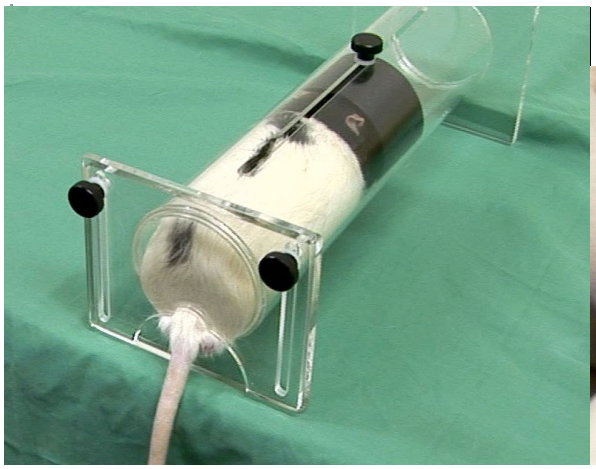
3. The needle is first directed slightly proximally to enter the group of muscles, then parallel to the femur to maintain the depth of the injection, while avoiding complete penetration of the muscle or touching the femur itself with the needle.
4. Care must be taken not to inject too deeply as it is possible to inject the sciatic nerve which runs caudal to the femur.
5. Gently draw back on the plunger, to ensure that no blood is drawn up into the syringe, and then inject the desired dose.

Intravenous Tail Vein Injection

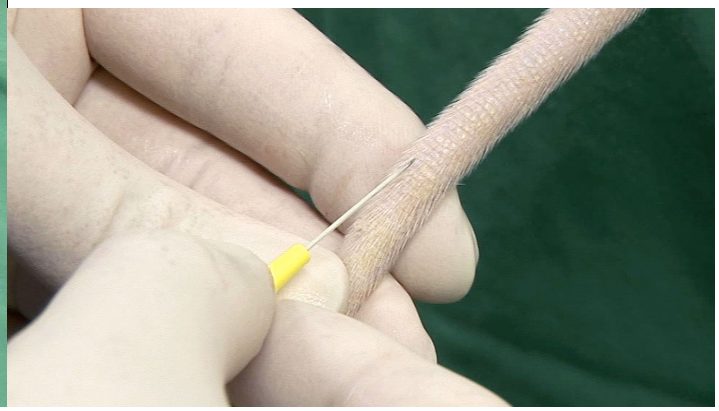
- Needle size 23 – 26G x 13 - 25mm, the NHMRC (2008) recommended maximum bolus volume is 1% of animal's body weight. If a larger volume is required seek the advice of the AWO or appropriate nominated/acting veterinarian.

- Anaesthesia may be used, depending on the advice of the AWO or appropriate nominated/acting veterinarian. If anaesthesia is not used then the rat may be restrained in a restraint device, see the photo below. Alternatively, the rat may be wrapped securely in a light towel and held by an assistant while the procedure is being performed. A light application of lignocaine cream to the venepuncture site 15 minutes before the procedure may reduce tail movement during the injection.

- The tail vein can be dilated by one of several ways:
  - (i) Occluding the vessel with the thumb and forefinger or a tourniquet.
  - (ii) Heating the tail by either immersing in warm water (37°C) for 1-2 minutes.
  - (iii) Placing the rat under a heat lamp, maximum wattage of 40, for 3-5 minutes.
  - (iv) Placing the rat in an incubator for 5 to 15 minutes at 30 to 35°C.
- 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. There are four blood vessels in the rat tail; the lateral (side) veins are used. Commence the injection as far caudal as possible on the tail.



Perspex rat restrainer



Lateral tail vein

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

Das RG and North D, 2007, Implications of experimental technique for analysis and interpretation of data from animal experiments: outliers and increased variability resulting from failure of intraperitoneal injection procedures, *Laboratory Animals*, 41, 312 – 320.

Genaro CA et al, 2007, Cecum location in rats and the implications for intraperitoneal injections, *Lab Animals*, Vol 36, No 7, 25 – 30.

Wolfensohn S and Lloyd M, 2013, *Handbook of Laboratory Animal Management and Welfare*, 4th ed, Wiley-Blackwell.

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

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