This document has been developed by The Australian National University's (ANU) Research Ethics Office. It has been endorsed by the ANU Animal Experimentation Ethics Committee (AEEC). It is designed to provide guidance regarding current best practice to institutional animal users and carers on the care and use of animals for scientific purposes. It has been prepared in consultation with the Australian code for the care and use of animals for scientific purposes 8th edition 2013 (The Code).

Document 004: Guidelines for Injection Techniques in Mice V2.0

1. Background

Injections in mice are often approved for the administration of compounds, drugs, antibodies, cells or other agents. The route of injection, frequency of injection and properties and volume of the substances must be carefully considered to minimise the impact on the welfare of the animal. As per the Section 3.3.7 of The Code:

When performing injections, blood sampling and non-surgical procedures, procedures used must:
1. minimise the risk of an animal developing complications (e.g. tissue damage, infection, haematoma, bleeding)
2. be performed under aseptic conditions if there is a potential risk of infection
3. if the procedure involves the transplantation of cells or tissues, include management of the effects of tissue rejection and immunosuppression.

Definitions

Adjuvant - substance which enhances the body's immune response to an antigen

Intragastric - given via gavage needle

Intradermal (ID) - administered into the dermal layer of the skin

Intramuscular (IM) - administered into the muscle

Intranasal (IN) - administered into the nose

Intratracheal (IT) - administered into the trachea

Intraperitoneal (IP) - administered into the abdomen for exposure by the peritoneal cavity and lymphatic system

Intravenous (IV) - administered through a blood vessel (vein) directly into the blood stream

Orally (PO) - per os **-** given by mouth directly or via food or water

Parenteral - administered by any route other than orally

Pyrogenicity - capacity to produce a fever or elevated body temperature

Subcutaneous (SC) - administered under the skin

2. General Information and Considerations

Route of Administration

The following factors should be considered when deciding on a route of administration for a given substance:

- pH (should be close to physiologic pH of 6.8-7.2), viscosity, concentration, sterility, pyrogenicity, biocompatibility and toxicity of the substance to be administered
- published data relating to the use of the substance to be administered and preferred routes of administration
- number of injections via the same route and the frequency of administration
- risks associated with the use of the substance
- risks associated with the use of sharps
- objective of the experiment

Ethical Considerations

In accordance with The Code, when performing injections, procedures used must:

- minimise the risk of an animal developing complications (e.g. tissue damage, infection, haematoma, bleeding)
- be performed under aseptic conditions if there is a potential risk of infection
- where a procedure involves the transplantation of cells or tissues, include management of the effects of tissue rejection, immunosuppression, the phenotype of the cells or tissues being transplanted and the potential effect on the recipient. Cell lines should be screened for pathogens before being injected into animals and the health status of source animal tissue/s should be assessed.
- be performed competently, and by a person who is competent for the procedures, or under the direct supervision of a person who is competent to perform the procedures
- cause the least harm, including pain and distress, to the animals.

When adjuvants are used to produce antibodies, the adverse impacts on animal wellbeing should be minimised by:

- using an adjuvant that provides an adequate antibody titre while causing the least adverse impact on the wellbeing of the animal
- using a ratio of adjuvant to antigen that reduces the probability of adverse reactions
- choosing the volume, site and frequency of injection/s of adjuvant that together optimise the antibody response and minimise the risk of complications occurring
- choosing a method and frequency of blood sampling that minimises the potential for harm, including pain and distress, to the animal.

When using non-pharmaceutical grade chemicals refer to the University's position papers:

- *Use of Non-Pharmaceutical Grade Discovery Compounds in Animals*

- *Use of Non-Pharmaceutical Grade Compounds for Anaesthetising & Euthanasing Animals*

3. Monitoring, Intervention and Reporting

Identification and monitoring

Prior to injections:

- animal transport needs to be undertaken in accordance with the 'ANU *Guideline for Animal Transport'* and acclimatisation periods observed where required
- animals must be clearly identifiable with the following information on cage cards and relevant computer programs or lab books available in the room housing the animals:
    - date and time of injection
    - injection type
    - amount injected
    - substance injected
    - initials of person injecting
    - monitoring requirements where applicable
- animals must be monitored before, during and after injections for signs of pain, distress or adverse events.
    - injections should not be performed on animals that are unwell unless justified (i.e. the injection is intended to improve the animal's condition or under the direct authorisation of a veterinarian).

Unexpected Adverse Events

If an unexpected adverse event occurs, the ANU *Procedure for Managing and Reporting Unexpected Adverse Events* must be followed. An unexpected adverse event is any event that may have a negative impact on any animal/s, and was not foreshadowed in the approved protocol or activity.

4. Minimum Requirements

Prerequisites for Injection

Unless otherwise specified in an approved ANU AEEC protocol, animals must meet the minimum age for the injection technique being performed.

The following prerequisites apply to the use of injection techniques in mice:

- ANZCCART ComPass Phase 1: core mandated training for animal users
- animal handling and cervical dislocation competency
- sharps safety competency
- competency in the ANU training for the rodent injection technique to be performed
- provision of a risk assessment for the procedure and substance(s) to be administered
- the procedure is being performed as per the approved AEEC protocol

- the person undertaking the procedure is listed on the protocol as competent or is a competent animal care technician

Loading Syringes

Except where otherwise stated in the approved AEEC protocol, the following requirements apply when loading syringes:

- one needle and syringe is to be prepared per animal to be injected
- the recapping of needles is not permitted
- substances should be stored in vials where possible to reduce the risks of aerosols
- bubbles must be removed from syringes to ensure injection accuracy
- workstations should be prepared with equipment for the disposal of sharps.

General Requirements

- Researchers must justify in their protocols the use of intramuscular (IM) injections in mice, which are not recommended due to their small muscle mass and potential for side effects such as paresis, necrosis, sloughing and irritation.
- Use 2-3 sites of administration when administering large volumes subcutaneously
- Researchers must note and justify in their protocol why a higher volume must be used if a dose volume falls between the ideal and maximum volumes (*Appendix III)* this needs to be noted in the protocol along with justification for the higher dose
- All substances which are given parenterally must be sterile.

5. Appendices

Appendix I: Intraperitoneal Injection

The use of IP injections is sometimes not the ideal method of drug delivery. It has been found that failure rates can be as high as 15% which can affect animal welfare and data quality. Investigators should also consider that IP injection is not a common route of drug administration in humans and therefore may not be suitable where translation of results is a priority. Alternative routes of administration, including subcutaneous or intravenous injections, may be better options. You can discuss your injection options with the ANU vets.

Unless otherwise specified in an approved ANU AEEC protocol, mice must be a minimum of six (6) weeks of age. Intraperitoneal injections should be undertaken in association with competence and experience in animal handling and restraint. Poor restraint can lead to complications such as:

- respiratory distress
- poor injection technique and increased incidence of adverse events
- increased risk of sharps safety incidents

Mice should be restrained using the non-dominant hand to allow for injection with the dominant hand. Mice should be tilted so that the head is lower than its hind end to allow

the abdominal viscera to shift towards the front of the body and to minimise risk of accidental puncture of organs.

Injections should be administered to the left or right of the midline. The needle should be inserted no higher than the knee of the hind leg paying attention to the animal welfare risks associated with poor technique. Some common issues with technique include:

- injecting too low which can cause injection into the fat pad and hence failure of the substance injected to be adequately absorbed. If an anaesthetic has been injected IP and the animal has not been anaesthetised as expected, care must be taken if providing a second dose as there may still be anaesthetic in the fat pad or similar area that can be slowly absorbed, thereby leading to overdose of the anaesthetic. Half or third doses are recommended and close monitoring of the animal until they are fully awake. The use of a reversal agent in these circumstances is highly recommended.
- injecting too shallow can result in subcutaneous injection and delayed absorption
- injecting too high or deep may result in organ or blood vessel laceration or injection into the gastrointestinal tract.
- unsteady hand, this may cause the needle to move around inside the mouse which may lacerate the organs.
- syringe position, ensure the gradations on the syringe can be read to determine the correct volume of solution to be injected.
- needle position, insert the needle bevel up on a 45° angle
- poor sterility which can result in infections of the peritoneal cavity (peritonitis)

Figure 1: Midline Identification

Figure 2: Injection location example

Appendix II – Intravenous Injection via the lateral tail vein

Unless otherwise specified in an approved ANU AEEC protocol, mice must be a minimum of eight (8) weeks of age. Intravenous injections should be undertaken in association with competence and experience in animal handling and restraint. Poor restraint can lead to complications such as:

- respiratory distress
- poor injection technique and increased incidence of adverse events
- increased risk of sharps safety incidents

Prior to injection

Mice may be heated in a warming chamber or under a heat lamp to dilate the lateral tail veins provided they are:

- not able to get burnt on the heat source
- only heated for approximately three (3) minutes (but no longer than five (5) minutes) in cohorts of five (5) or less. (This will be dependent on variables such as the intensity of the heat source, how close it is to the mice, the time taken to inject the animals and the proficiency of the technician)
- constantly monitored for signs of heat stress including increased burrowing behaviours, rapid breathing, reduced activity/lethargy or salivation. If any of those behaviours are displayed; mice must be removed from the heat source immediately regardless of whether the minimum timeframe has elapsed.
- never reheated within an injection session.

During Injections

Mice must be restrained using an approved manual restraint device. Devices must provide safe and secure restraint for access to the lateral tail veins while ensuring that mice have the ability maintain clear airways for breathing. Mechanical restraints must be approved for use in the applicable ethics application, be clean, in good working order and must be cleaned between individual animals. Intravenous injection is conducted via the lateral tail veins as per Figures 3 and 4; paying attention to the animal welfare risks associated with poor technique. To ensure good technique:

- the needle should be held parallel to the vein with the bevel facing up
- avoid injecting into the surrounding tissue, and inject slowly into the vein. Resistance when attempting to inject may indicate that the needle is not in the vein
- no more than two attempts are to be made in each vein. If the first attempt is unsuccessful, another attempt may be made on the same vein in a more proximal (higher) position on the tail.

Where subsequent attempts are required, check the needle for dry blood that may be causing a blockage and gently expel the blood if required. Change the needle for a new one if required.

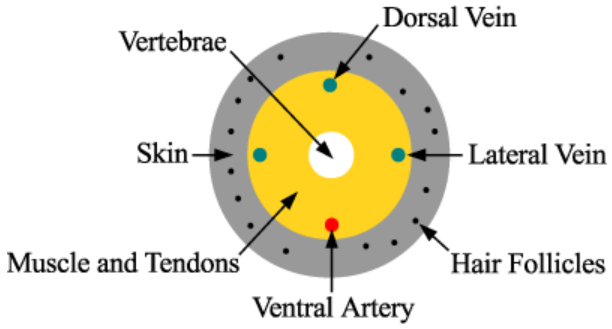

Figure 3: Mouse tail cross section with blood vessel locations.

Appendix III –Maximum Injection Volumes (per adult animal)

Species	PO gavage	IV	IP	SC	IM	IN/IT*	ID*
Mouse	<200ul (10ml/kg)	<200ul	<200ul (10ml/kg)	<500ul	<20-50ul	<50ul	<50ul
Rat	<10ml/kg	<10ml/kg	<10ml/kg	<10ml/kg	<200ul	<50uL	<50ul

Figure 4: Abbreviations: (PO)- orally- given by mouth alone or in food or water, (gavage) given via a gastric gavage needle for rodents or stomach tube for larger species, (IV)- intravenous administered through a blood vessel directly into the blood stream, (SC) subcutaneous- administered under the skin, (ID)- intradermal- administered directly into the dermal layer of skin, (IM) – intramuscular - administered into the muscle, (IP)- intraperitoneal - administered into the abdomen for exposure by the peritoneal cavity and lymphatic system, (IN)- intranasal - administered into the nose, (IT)- intranasal -administered into the trachea. Maximum volumes for each method in brackets next to idea volume.

*Anaesthesia may be required for these techniques

Appendix IV – Recommended locations and needle size for injection of substances by site

	Preferred site of injection	Maximum needle size
PO/ gavage	Oral, intragastric via gavage needle	<20 gauge (adult mice) <24 gauge (young mice)
IV	Tail vein, saphenous vein	25-29 gauge (mouse) 21-26 gauge (rat)
IP	Lower right abdominal quadrant	25-26G gauge (mouse) 25-27g (rat)
SC	Intrascapular, neck, shoulders, flank, lower back	25 gauge

IM	Quadriceps muscle (only if no other alternative methods of injection available)	27 gauge (mouse 25 gauge (rat)
IN	Nose	<22 gauge or 100µL pipette tip
IT	Trachea	<22 gauge
ID	Skin	<25 gauge

Figure 5: Recommend sites for injection

6. References and Resources

ANU Position Paper: Use of Non-Pharmaceutical Grade Discovery Compounds in Animals https://services.anu.edu.au/research-support/ethics-integrity/animal-ethics-policies-guidelines-and-forms

ANU Position Paper: Use of Non-Pharmaceutical Grade Compounds for Anaesthetising & Euthanasing Animals https://services.anu.edu.au/research-support/ethics-integrity/animal-ethics-policies-guidelines-and-forms

Procedure for Managing & Reporting Unexpected Adverse Events https://services.anu.edu.au/research-support/ethics-integrity/animal-ethics-policies-guidelines-and-forms

NHMRC. Australian code for the care and use of animals for scientific purposes 8th Edition 2013 (Section 3.3.7) https://www.nhmrc.gov.au/about-us/publications/australian-code-care-and-use-animals-scientific-purposes

IACUC Indiana University. IACUC Policy for Dose Volumes in Laboratory Animals 2017 https://research.iu.edu/policies/iupui-animal-care-and-use.html

OACU, National Institutes of Health. Mice injection routes, maximum needles sizes & volumes 2021 https://oacu.oir.nih.gov/nih-policies

Turner, P.V., Brabb, T., Pekow, C., Vasbinder, M.A. (2011) Administration of substances to laboratory animals: routes of administration and factors to consider, *Journal of the American Association for Laboratory Animal Science,* 50 (5), 600-613.

UBC. Animal Care Guidelines: Intraperitoneal (IP) injection in rats and mice SOP 2014 https://animalcare.ubc.ca/animal-care-committee/sops-policies-and-guidelines/standard-operating-procedures