1. Background

Injections in mice are often approved for the administration of compounds, drugs, antibodies, cells or other agents.

2. Considerations

Route of Administration

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

- pH, viscosity, concentration, sterility, pyrogenicity 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
- 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, procedure involve 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
- 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 of adjuvant that together optimise the antibody response and minimise the risk of complications
- choosing a method and frequency of blood sampling that minimises the potential for harm, including pain and distress

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 should 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 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 animals 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:

- ANML03 Animal Ethics Seminar
- 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 person undertaking the injection is approved to do so under the relevant approved AEEC protocol

Loading Syringes

Except in approved circumstances such as in an approved and current ethics protocol or amendment, 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
Appendix I: Intra peritoneal Injection

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. 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 failure to be adequately absorbed
- injecting too shallow can result in subcutaneous injection and delayed absorption
- injecting too high or deep may result in organ 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

![Figure 1: Midline Identification](image1.png) ![Figure 2: Injection location example](image2.png)
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 burn themselves on the heat source
- only heated for approximately three minutes (but no longer than five minutes) in cohorts of five 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 a safe and secure restraint for access to the lateral tail veins while ensuring that mice have the ability maintain clear airways. 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, 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.

![Figure 3: Mouse tail cross section with blood vessel locations.](image-url)
6. References and Resources

The Australian code for the care and use of animals for scientific purposes 8th edition. 2013
Procedure for Managing & Reporting Unexpected Adverse Events
ANU Position Paper: Use of Non-Pharmaceutical Grade Discovery Compounds in Animals
ANU Position Paper: Use of Non-Pharmaceutical Grade Compounds for Anaesthetising & Euthanasing Animals