LAB_028 Injections - Intra-peritoneal (IP) in Mice, Rats and Neonates (Expires May 2027)

I. OBJECTIVE

To describe the standard IP injection procedure in mice and rats used across UQ research projects, also reflecting the procedure used to train workers across UQ by UQBR.

NB: The use of (*) indicates this statement is dependent on the facility procedures
NB: The use of (**) indicates this statement is dependent on AEC Approvals

II. DEFINITIONS

Competent - "the consistent application of knowledge and skill to the standard of performance required regarding the care and use of animals. It embodies the ability to transfer and apply knowledge and skill to new situations and environments."[1]
Intraperitoneal – situated within or administered by entering the peritoneum

III. Considerations when using this SOP in ethics applications

- You should state the volume to be injected in your application. See the table below for maximum volumes.
- If you would like to perform more than 5 IP injections per mouse, provide justification to the AEC in your application
- The frequency of intraperitoneal injections should be as low as possible. If more than one IP injection per week is required, please provide justification to the AEC in your application

IV. Choice of IP instead of other routes of administration

- It is important that you consider the best route of administration for any substances
- There is evidence to show that IP injection is an unreliable route for administration of substances in rodents as injection may inadvertently enter the caecum, fat, subcutaneous tissue or organs.
- Ideally, IP injection should only be used where other routes are unsuitable. If using IP injections in a project, it would be ideal to justify why IP is more suitable than another route such as subcutaneous.

V. COMMENTS / RECOMMENDATIONS

- Intraperitoneal injection must be performed by appropriately trained personnel who have been deemed to be competent in the procedures.
- A maximum of 1% of total body weight can be injected where the aqueous solution is rapidly absorbed, this must be reduced for oily solution as distension of the abdomen is painful.
- **Aseptic technique** should be used in making up solutions, dilution of substances, drawing up the substance and injecting the animal. This includes using a new needle for each animal.

[1] NHMRC, 2013, *Australian code for the care and use of animals for scientific purposes*, National Health and Medical Research Council (NHMRC).

- **Clean technique** should be used in preparing the skin i.e. make sure skin is clean and dry. Wipe with 70% ethanol or similar if this is appropriate for the substance being injected.
- **Aseptic technique** should be used to prepare the skin if there is a risk of infection. For example, when injecting tumour cells, biologicals or jells. For example, clip the hair, clean with antiseptic such as chlorhexidine or betadine, wipe with 70% ethanol.
- After drawing up a substance, a new needle should be used to inject the animal. This is to ensure a sharp needle and minimise contamination.

Neonate comments

- For neonates consider using low volume syringes to improve volume accuracy when performing in neonates
  A 0.3 mL insulin syringe is ideal for this work, it will allow the substance to be injected at a steady pace.
- Handling pups may change their smell, where possible encourage mother to mark pups
  You can also rub your gloved hands in the dirty bedding in the cage before restraining, this will allow the smell to transfer to your gloves.
- Ideally select pups that have recently fed by identifying a prevalent milk spot. There is a possibility pups may not feed soon after procedures
- Ensure holding cage has heat source provided until the animal is able to access the mother.
- Any unexpected loss of pups must be considered as an adverse event, these animal numbers are included in animal usage counts

Calculation of injection volume

It is vital that the correct volume to be injected is calculated. Injection volume is generally calculated as a % of total body weight. This is the clinically relevant value.

Example calculation to obtain 1% of the total weight of a 20 gram mouse

0.01 x 20 = 0.2 mls or 200 µl

The table below provides some examples

Table 1. Recommended values for Neonate, Mice and Rats IP Injections (NHMRC 2008)

Values	Neonate	Mouse	Rat
Needle Gauge	31-29G	25-27G	23-26G
Needle Length	12mm	13-25mm	13-25mm
Needle Depth at Injection	0.25cm	0.5cm	1cm
Volume to be injected	Only inject the volume approved by the AEC		
Max Injection Volume	25uL	1% of bodyweight in a bolus injection	1% of bodyweight in a bolus injection
Attempt allowance	One attempt. If unable to inject, allow another trained and competent person on the project to complete.		
Procedure number and frequency	The minimum number possible.		

VI. SAFETY AND COMPLIANCE

1. The person undertaking this task must ensure all relevant approvals are in place, training has been undertaken and risk assessments have been performed. If unsure, consult your supervisor.
2. Facility protocols should be followed.
3. Possible risks include mouse bite injury, needle stick injury, spills, exposure to infectious agents, repetitive task musculoskeletal injury and psychosocial harm.

VII. TRAINING CONSIDERATIONS

- All unsupervised animal injections must be performed by appropriately trained personnel who have been deemed to be competent in the procedure.
- Training for injections collection must be undertaken on models or cadaver animals initially.
- Note for UQBR Training purposes, 1% of total body weight may be injected in a bolus injection.

- For UQBR training purposes animals may remain for a number of days to monitor. Adverse effects may take time to develop and can assist with the assessment of competency.

VIII. EQUIPMENT

- PPE *
  Minimum PPE is gloves and gown, additional PPE may be required based on facility or additional risk e.g. working with infectious animals.
- Disinfectant *
- Sharps Container
- Clinical waste bin
- Syringe
- Needle (Refer to table 1 for needle gauge and length)
- Substance for Injection**
- Change station/Bio-safety cabinet *

IX. PREPARATION

1. Check AEC approvals to ensure that the correct procedure and personnel are approved for the planned work
   Deviations can occur between approved procedures listed versus what is planned with the animal – check that these match and that the relevant personnel are approved.
2. Set up equipment items
   There should be no contamination of needles or substance for injection during this process.
3. Turn on Change station or Biosafety Cabinet *
4. Wipe surfaces with disinfectant
   Ensure equipment is operating as required.

X. PROCEDURE

Preparation of Injection Substance

Refer to UQBR Online Module for Needle Safety

- Confirm the concentration and volume with the approved AEC protocol
  The injectable solution volume is limited to 1% of total body weight.
  Consider temperature, pH, injection of cells, hazardous substances (cytotoxic, radioactive, infectious), and highly viscous liquids to improve success of procedure. These considerations can impact safety and animal welfare, refer to Reference Information below for information about these variables.

- It is the responsibility of the researcher to convey all risks associated with compounds and materials to be used. This may include lab specific risk assessments and SDS and other OHS obligations.
  If substances to be used are experimental or off label (i.e. no Safety Data Sheet is available), the laboratory is responsible for conveying all of the risks to workers involved in the project. This includes risk of performing the

procedure as well as the risks associated with animal husbandry such as waste management of cage bedding and cadavers that UQBR staff may be exposed to. Exposure maybe acute or chronic.

Preparation for Restraint

Mouse Restraint for IP Injection Refer to LAB_039 Handling and Restraint in Rats and Neonates and LAB_006 Handling and Restraint in Mice and Neonates

- When performing IP Injections angle the body 45° toward the ground (head down). This will assist with the needle angle and create more space in the peritoneal cavity to inject into. However, this will not eliminate the risk of injecting into an organ. See the considerations section at the start of this SOP.

Neonate Restraint for IP Injection Refer to Figure 3. Position and injection site for IP injections in Neonates

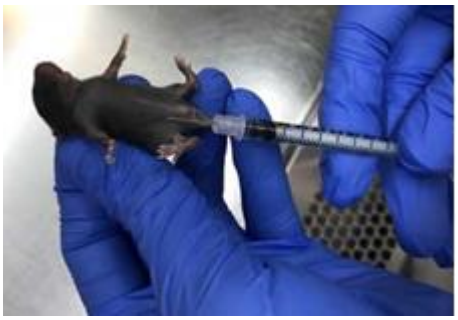

Figure 1 Neonate restraint

Rat Restraint for IP Injection – Technique 1 Refer to LAB_039 Handling and Restraint in Rats and Neonates and LAB_006 Handling and Restraint in Mice and Neonates

- Restrain animal in sternal recumbancy and use the tail base to lift up the pelvis up at a 45º angle, or lift the hind leg away from the abdominal cavity
- Inject into the caudal peritoneal cavity on either side of the midline

Rat Restraint for IP Injection – Technique 2 Refer to LAB_039 Handling and Restraint in Rats and Neonates and LAB_006 Handling and Restraint in Mice and Neonates

- Restrain the animal using either the cross over or claw grip method and hold the tail with the other hand. Lower the animals head.
- Inject into the caudal peritoneal cavity (or a second person may inject) on either side of the midline

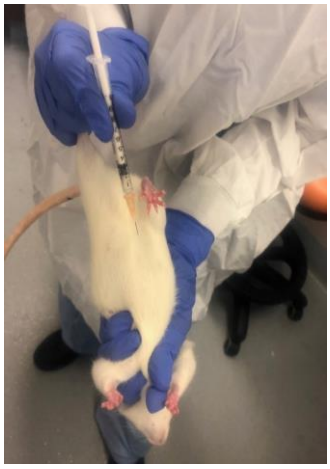

Figure 2. Restraint Technique #2 in the rat

IP Injection Procedure

1. Have your needle ready with the solution you need to inject drawn up.
   Ensure there are no air bubbles present in the syringe, these can be removed by pulling up and down on the plunger drawing the solution back and forward slowly. The needle should be uncapped and placed appropriate location until used as per Needle Use and Sharps Safety training.

2. Identify animal to be injected – *check animal's identification marks*

3. Restrain the rodent based on the species and age for specific technique
   Be sure to hold enough skin so the animal cannot bite or kick. Movement of the animal during the procedure can cause needle stick injuries or misplaced injection.

4. Locate the midline of the animal and the top of the hind leg, the injection site will be halfway between these 2 points, preferably the rodent's right peritoneal region is to be used
   The midline is the line in the fur that runs down the center of the animal's body. Using the lower right quadrant of the abdomen will help you avoid the caecum and bladder. However, when multiple injections are required it is essential to alternate sides.

5. Holding the syringe in your dominate hand, insert the needle bevel up at a 30-40 degree angle at a depth dependent on the rodent's size/age
   For a 20-25G mouse the depth of entry will be approximately 0.5cm. Upon entry it is the most likely time for the animals to react. Be sure to have a steady hand as moving the needle around can lacerate organs.

6. Inject pre-determined volume as per AEC approval

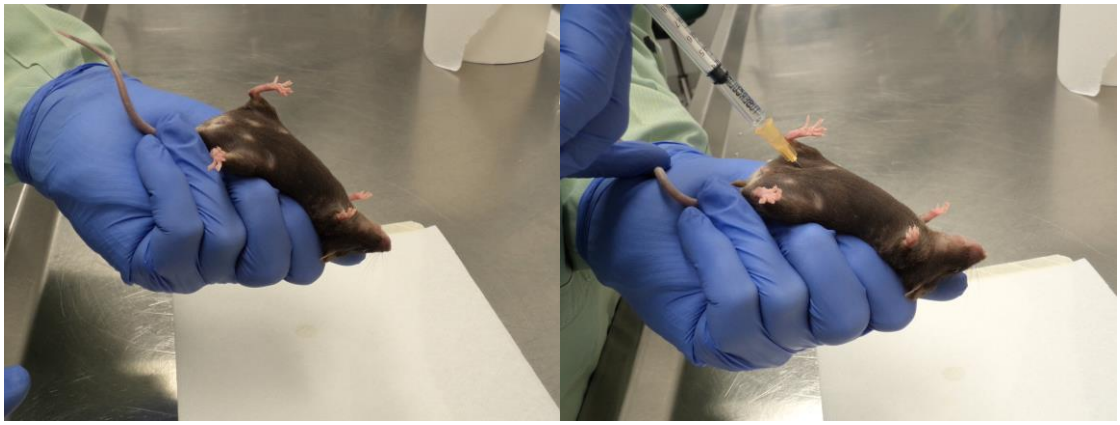

Figure 3. Position and injection site for IP injections in adult mice

Figure 4. Position and injection site for IP injections in Neonates (UQBR 2019)

7. Pause for a couple of seconds to eliminate the risk of leakage and then remove the needle slowly

8. Release the rodent into holding cage and continue to monitor for recovery and health
   Following the procedure, the animal should be monitored for any potential complications. For example, if you see the animal hunched, irritable, or change in demeanour. If concerned refer to the LAB_022 Veterinary Care Program.

9. Place needle into sharps container and syringe into clinical waste bin **
   Always use the specialised needle remover located on the lid of the sharps bin, if this cannot be located place the needle and syringe in the sharps bin as one unit. A new needle should be used for each animal.

10. Complete record keeping requirements – a record should be kept of the procedure, date, side of abdomen, substance, who performed it, and any complications. This record can be kept anywhere appropriate that conforms with the animal code and UQ research policies. A health alert cage card (or similar) needs to be placed indicating which procedure was performed to ensure the animal is monitored carefully for a minimum

of at least 2 days. If the study is blinded, the cage card does not need to include substances etc. Record keeping may include UQBR records, lab book, AEC animal monitoring paperwork and the relevant research sample collection labelling/records.
Injection procedures should also include the substance and volume injected. Records need to be clear and legible on each record to allow others to read and understand.

11. Repeat these steps for the next animal or if finished, pack and clean up equipment and space.

XI. REFERENCE INFORMATION

Injection Considerations
Accuracy - One study has referenced up to 17% failure rate of IP injection completed by trained and licensed individuals (Ballard 2009). Generally, this is due to accidental dosing into the caecum, depending on the research this is a consideration for expected outcomes.

Temperature – Consider if the substance has been stored in the fridge, if possible, allow it to reach room temperature before injecting into the animal due to comfort and possible impact on body temperature.

Experimental Substances – A need for increased monitoring is generally required for experimental substances

Non-biological pH – There are mechanisms to improve pH of a substance for injection. For example, increasing the dilution, change of delivery vehicle etc. This can decrease the risk of internal tissue necrosis and improve procedure outcomes.

If the substance is not a neutral pH of ~7, it may be acidic or alkaline, replace the needle that was used to draw up the solution before injection to decrease any pain on entry to the animal. If a pH is likely to cause pain on injection, it is ideal to anaesthetise the animal.

Radioactive Substances – Additional approvals and safety precautions are required and will be included in the risk assessment. It is common to require safety goggles, additional gloves and shielding. You may also be required to work under a licensed person.

Infectious – Additional approvals and safety precautions are required and will be included in the risk assessment. Additional training may be required to ensure containment of infectious agents and waste management to protect other research projects and human health.

Cytotoxic – Additional approvals and safety precautions are required and will be included in the risk assessment. Additional training may be required to ensure containment of cytotoxic agents and waste management to protect other research projects and human health.

Non-TGA approved and off label substance use – If substances are experimental there may not be an SDS available. Ensure the risk assessment for the use and management of the substance includes excretion of the substance from the animal, chronic versus acute exposure, waste management of bedding/cage handling.

Injecting Schedule 7, 8 or 9's – The use and possession of these scheduled drugs requires special QLD Health Approval. Please ensure you have QLD Health 'Researcher Approval to 'possess', 'use' and 'dispose' of these drugs during project planning. Seek further advice about this from UQBR or your local area Drugs Officer.

UQBR Training – For UQBR training purposes animals may remain for a number of days to monitor. Adverse effects may take time to develop and can assist with the assessment of competency.

XII. BIBLIOGRAPHY

1. Ballard, T 2009, 'Intraperitoneal route of administration – how accurate is this technique?', Animal Technology and Welfare, vol. 8, no. 1, pp. 17-18.
2. National Health and Medical Research Council (NHMRC) 2008, *Guidelines to promote the wellbeing of animals used for scientific purpose,* viewed 11 April 2019, https://www.nhmrc.gov.au/about-us/publications/guidelines-promote-wellbeing-animals-used-scientific-purposes

Version #	Reviewing AEC (note: all other relevant AECs ratify the approval)	AEC Review Date	Approval To Date
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