 <p>THE UNIVERSITY OF QUEENSLAND AUSTRALIA CREATE CHANGE</p>	<p>UQ Animal Ethics Committee - Standard Operating Procedure LAB_028 Injections - Intra-peritoneal (IP) in Mice, Rats and Neonates Institutional author: UQ Biological Resources AEC Reviewed & Approved: 13th Feb 2020</p>	Version 4
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LAB_028 Injections - Intra-peritoneal (IP) in Mice, Rats and Neonates

I. OBJECTIVE

To describe the IP injection procedure in mice and rats that is used within UQBR facilities.

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. SAFETY

1. This procedure has the risk of needle stick or mouse bite injury – take appropriate care.
2. This procedure has a risk of causing musculoskeletal injury when performed regularly – consider suitable ergonomic design whenever possible.
3. In the event of a spill follow the facility emergency spill procedure.
4. Ensure you are familiar with the SDS for the substance to be injected should exposure or spills occur
5. Splash back into the face or eyes are a risk of performing injections. Protective visors or safety goggles should be worn at all times during the procedure

III. 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 **
- Substance for Injection**
- Change station/Bio-safety cabinet *

IV. PREPARATION OF EQUIPMENT

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.

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Aseptic Technique

Use an aseptic technique when performing procedures, this will minimise contamination from pathogens and subsequently infection in research animals.

V. PROCEDURE

Preparation of Injection Substance

Refer to UQBR Online Module for Needle Use and Preparation.

- Confirm the concentration and volume with the approved AEC protocol
The NHMRC Guidelines for a bolus IP injection volume is 1% of total body weight, any volume larger than this should be clearly cited and justified in the AEC application. For example, a 25g mouse may have up to 250ul injected IP.
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.
- Unless specific directions are provided in the AEC approved project, refer to NHMRC Guidelines for recommended maximum injectable volumes and recommended needle gauge.
The maximum needle gauge is outlined in the NHMRC Guidelines. Refer to Reference information below for guidance.
- 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_006 Handling and Restraint of Mice and Rats

- When performing IP Injections angle the body 45° angle 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 'Injection considerations section at the end of this SOP.

Neonate Restraint for IP Injection

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

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Rat Restraint for IP Injection – Technique 1

Refer to LAB_006 Handling and Restraint of Mice and Rats

- Restrain animal in sternal recumbancy and use the tail base to lift up the pelvis up at a 45° angle
- Inject into the caudal peritoneal cavity on either side (preferably left) of the midline

Rat Restraint for IP Injection – Technique 2

Refer to LAB_006 Handling and Restraint of Mice and Rats

- Restrain the animal in lateral recumbancy and lift the top hind leg away from the abdominal cavity
- Inject into the caudal peritoneal cavity

Rat Restraint for IP Injection – Technique 3

Refer to LAB_006 Handling and Restraint of Mice and Rats

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

Rat Restraint for IP Injection – Technique 4

Refer to Figure 1. Restraint Technique #4 in the rat (UQBR 2019)

Refer to LAB_006 Handling and Restraint of Mice and Rats

- Restrain the animal using either the cross over or claw grip method and hold the tail and one hind leg in the other hand.
- Inject into the caudal peritoneal cavity on either side (preferable left) of the midline

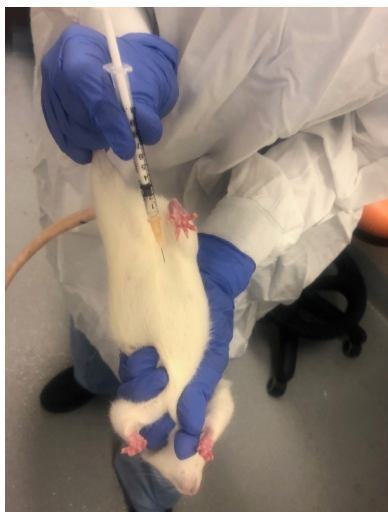


Figure 1. Restraint Technique #4 in the rat (UQBR 2019)

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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 half way 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. On entry point it is the most likely time for the animals to kick or bite. Be sure to have a steady hand as moving the needle around can lacerate organs.
6. Inject pre-determined volume
Volume for injection is as per the animal ethics committee approved activity.

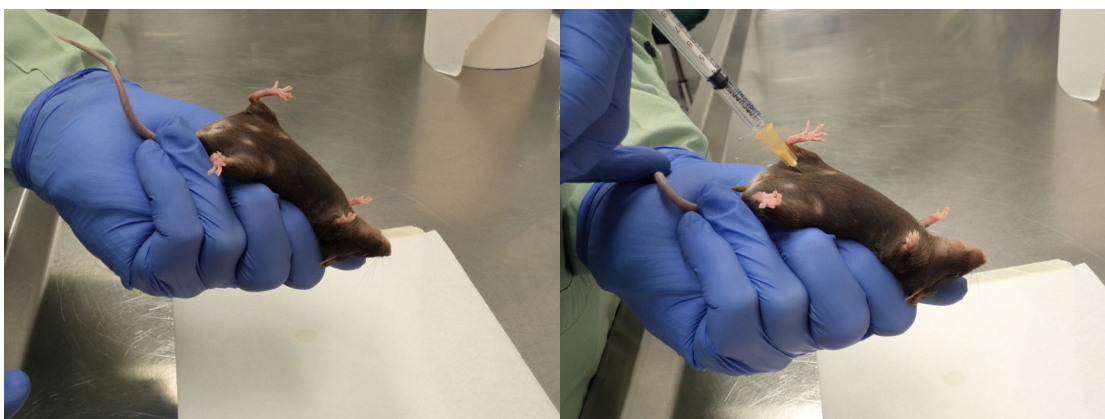


Figure 2. Position and injection site for IP injections in adults mice (UQBR 2019)

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Figure 3. 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
The animal's fur should be free of blood and injection fluid, ensure there are no cuts or scratches around the injection site. If you do see blood a small amount of pressure should be applied with clean gauze until the bleeding ceases.
8. Release the rodent into holding cage and continue to monitor for recovery and health
Following the procedure, the animal should recover to normal movement once placed back in the cage, if you see the animal tucking the abdomen up, jumping once in their home cage or excessive cleaning of the area this could be an indication the rodent is in pain. Seek veterinary advice. If discomfort is observed refer to the UQBR SOP 22 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 – note procedure, date and initials on cage card, log procedure on relevant 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.

Considerations for Neonates

- 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.

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- 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. Refer to Online Heating Module
- Note any unexpected loss of pups must be considered as an adverse event. Note these animal numbers are included in animal usage counts.

VI. Reference Material

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	12-12mm	13-25mm	13-25mm
Needle Depth at Injection	0.25cm	0.5cm	1cm
Max Injection Volume	25uL	1% of bodyweight in a bolus injection	1% of bodyweight in a bolus injection

UQBR Training Consideration

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.

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

Cells – When injecting cells, a larger gauge needle may need to be used. In a mouse a 25g needle will safely inject most cells. Depending on the research there may be a need to handle the needle and syringe in a specific manner for successful cell delivery.

Non-biological pH – There are mechanisms to improve pH of a substance for injection. For example, increasing the dilution, change of delivery vehicle, or anaesthetising the animal. 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 drawn up the solution before injection to decrease any pain on entry to the animal.

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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.

VII. REFERENCES

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-usedscientific-purposes>
3. Office of the Gene Technology Regulator (OGTR) n.d., viewed 11 April 2019, <http://www.ogtr.gov.au/>
4. University of Queensland n.d., *Health, safety and wellbeing*, viewed 11 April 2019, <https://staff.uq.edu.au/information-and-services/health-safety-wellbeing>
5. University of Queensland n.d., *Incidents, injuries and hazard*, viewed 11 April 2019, <https://staff.uq.edu.au/information-and-services/health-safety-wellbeing/health-safetyworkplace/incidents-injuries-hazards>
6. UQ Biological Resources n.d., *UQBR SOP's*, viewed 11 April 2019, <https://biologicalresources.uq.edu.au/secure/reference-information#SOP's>
7. UQ Biological Resources, 2019 *IP Injections*.

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