

	UQ Animal Ethics Committee - Standard Operating Procedure LAB_042 Injections - Intrahepatic Injection in Neonates Institutional author: UQ Biological Resources AEC Reviewed & Approved: March 2025 SOP Expiry: March 2026	Version 2
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LAB_042 Injections - Intrahepatic Injections in Neonates (Expiry: March 2026)

I. OBJECTIVE

To describe the intrahepatic (IH) injection method in neonates used within the 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 rodent 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.
6. Ensure you are familiar additional risk assessments or standardised care of animals associated with being Irradiated and any radiation safety and protection plans approved by Radiation Health QLD.

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
- Insulin Syringe (30g)
- Substance for Injection**
- Change station/Bio-safety cabinet *
- Small holding container / dish
- Heat source with ongoing monitoring ** E.g. Heat mat
- Sterile Swabs
- Tissue (Kimwipes®)

Conditions:

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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. Prepare equipment items
There should be no contamination of needles or the injection substance during this process.
3. Turn on Change station or Biosafety Cabinet *
4. Wipe surfaces with disinfectant
Ensure equipment is operating as required. Consider using benchcoat if there is a need to place the pup on the work surface.

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.

1. Confirm the concentration and volume with the approved AEC protocol **
Volumes may vary with age of neonates this should be clearly cited and justified in the AEC application.
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.
2. Unless specific directions are provided in the AEC approved project, refer to NHMRC Guidelines for recommended maximum injectable volumes and recommended needle gauge.
Using an insulin syringe with affixed 30g needle is recommended. Changing your needle for each injection is recommended.
3. 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 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.

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Preparation for Restraint for IH Injection

Refer to LAB_006 Handling and Restraint in Mice and Neonates

Refer to LAB_039 Handling and Restraint in Rats and Neonates

1. Grasp loose skin behind the neck. The skin along the back may also be grasped when the neonate is large enough.

This should be enough skin to gently immobilise the neonate. When restraining ensure the skin across the chest is not too tight. Loosen the skin held to provide relief. If you notice the slowing of movement or consciousness, immediately release the rodent.

Consider odour transferred from gloves, where possible use bedding from the home cage rubbed on fingers before handling the pup to minimise stress during handling. Textured gloves are recommended to form a better hold of the neonate to control movement. Restraint of neonates for injection is below (Figure 1), alternatively the legs can be placed behind your finger to reduce movement.



Figure 1. Restraint of neonate for IH injection (UQBR 2020).

Intrahepatic Injection Procedure

1. Have your syringe and needle ready with the solution 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 in an appropriate location until used as per Needle Use and Sharps Safety training.

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 THE UNIVERSITY OF QUEENSLAND AUSTRALIA CREATE CHANGE	UQ Animal Ethics Committee - Standard Operating Procedure LAB_042 Injections - Intrahepatic Injection in Neonates Institutional author: UQ Biological Resources AEC Reviewed & Approved: March 2025 SOP Expiry: March 2026	Version 2 Page 4 of 6
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2. Identify animal to be injected.
Check animal's identification marks.
3. Locate the milk spot in the stomach, if visible in the upper left abdominal quadrant.
Prior to injection check if neonates have a visible milk spot on abdomen, Figure 2 shows the location of the milk spot.
4. Restrain the neonate
Movement of the animal during the procedure can cause needle stick injuries or misplaced injection.
5. Locate the liver in the central umbilical quadrant of the abdomen.
This usually is visualised as a dark red elongated spot under the skin. The location of the liver can be visualised along with the milk spot (Figure 3). Ensure neonates are not exposed to the air flow of the biosafety cabinet as this can dry the skin and drop body temperature making it harder to visualise the liver.
6. Holding the syringe in your dominant hand, insert the needle at a depth of 2-5mm into the liver perpendicular to the body (Figure 4).
The depth may be dependent on the size and age of the neonate at time of injection. Take care during injection as there will be no resistance other than skin puncture, it is possible to go through the liver resulting in a misplaced injection.

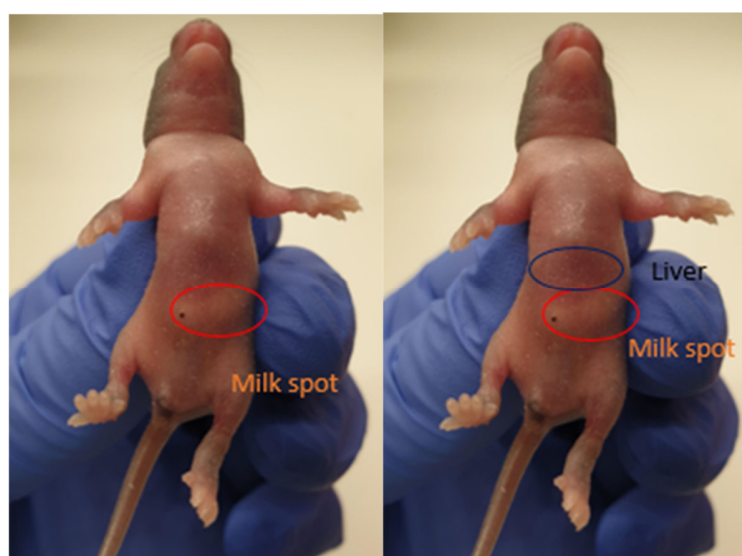


Figure 2. Location of milk spot and the liver (UQBR 2020)

7. Inject pre-determined volume slowly.
Volume for injection is as per the animal ethics committee approved activity. For UQBR training purposes a maximum volume of 50 μ l will be injected.
8. Wait 3-5 seconds after the injection is complete, then slowly and smoothly remove the needle.

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When the injection is completed, allow the needle to drop slightly under its own weight to stop blood/injectate from leaking out of the injection site. There should be no cuts or scratches around the injection site. If there is leakage (e.g. > 5ul) of the substance immediately stop the injection and alter injection site, the tip of the needle may not be within the skin layer.

9. Place neonate into the home cage continue to monitor recovery and health.
Minimise the time neonates are separated to decrease stress in both mother and pups. Use nesting and bedding material from the home cage to transfer the smell to the pups. If discomfort is observed refer to the UQBR SOP 22 Veterinary Care Program. Consider if the pup needs to be identified before placing into the home cage (AEC approval required).
10. 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.
11. 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.
12. Repeat these steps for the next animal or if finished, pack and clean up equipment and space.

VI. REFERENCE INFORMATION

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

Tumorigenic compounds - This technique is only suitable for non-abrasive solutions and the pH should be identified and considered appropriate.

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.

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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.

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.

VII. REFERENCES

1. 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>
2. Office of the Gene Technology Regulator (OGTR) n.d., viewed 11 April 2019, <http://www.ogtr.gov.au/>
3. University of Queensland n.d., *Health, safety and wellbeing*, viewed 11 April 2019, <https://staff.uq.edu.au/information-and-services/health-safety-wellbeing>
4. 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>
5. Journal of Visual Experiments (JOVE) 2019, *Chronic, Acute and Reactivated HIV infection in Humanised Immunodeficient Mouse Models*, viewed 5 June 2020, <https://www.jove.com/video/60315/chronic-acute-reactivated-hiv-infection-humanized-immunodeficient>
6. UQ Biological Resources, 2020 *UQBR Image Library*.

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