LAB_044 Injections – Tattooing in Neonates

I. OBJECTIVE

To describe the injection method used to tattoo neonates for identification purposes 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
- Change station or Biosafety Cabinet
- Needle (25-31 G) **
- Substance for injection**
  For tattooing purposes on human grade tattoo ink should be used

IV. 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. Disinfect tools that will contact the animals, or sterilisation where relevant.

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 **
  Generally a maximum of 1-2ul is advised per site unless otherwise approved by the AEC.

- 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.

Restraint for Injection

Refer to LAB_006 Handling and Restraint of Mice and Rats

1. When performing this Injection angle the needle almost parallel to the foot.

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 holding the syringe upright then pulling up and down on the plunger drawing the solution back and forward slowly. Tattoo ink is highly viscous, consider drawing up the solution without a needle on the syringe. Fill the syringe to no more than 0.5 mL to improve control over the needle and syringe during injection. The needle should be uncapped and placed in the appropriate location until used.

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

3. Restrain the rodent based on the species and age for specific technique
   Within UQBR animals are tattooed between 6 to 14 days old without anaesthesia.
   Be sure to restrain at the ankle/wrist to expose the bottom of the paw or foot so the animal cannot bite or kick. Movement of the animal during the procedure can cause needle stick injuries or misplaced injection.

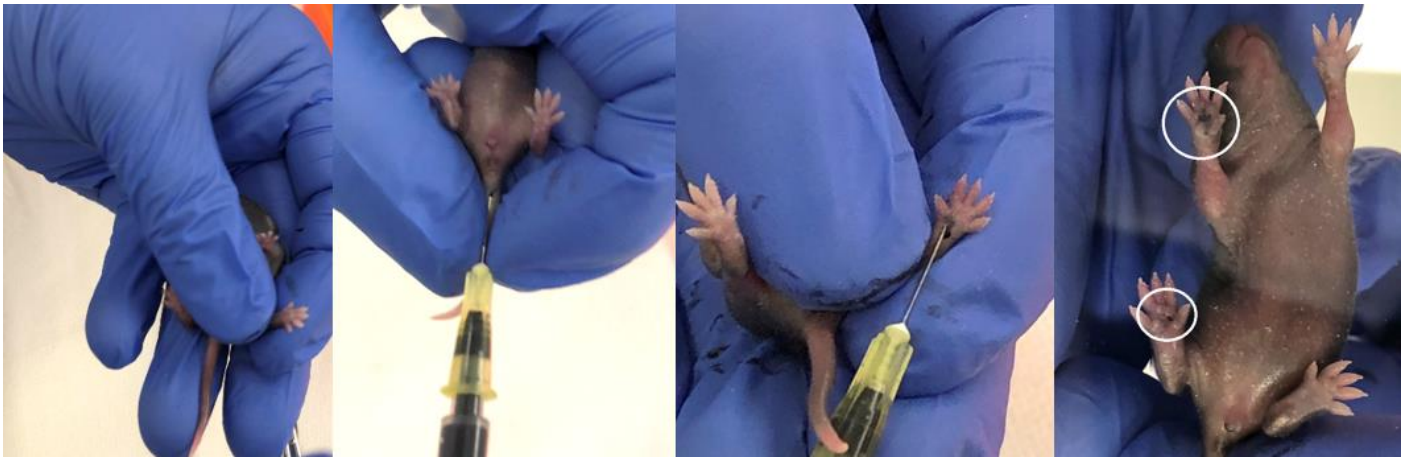

Figure 1 Restraint and injection landmarks into the base of tail and foot pad (UQBR 2020)

4. Holding the syringe in your dominate hand, insert the needle bevel up and at a depth where the full bevel of the needle is just under the skin. The injection is made underneath the rodent's front paw, hind foot, or base of tail.
   Locate the pedal vein that is midline of the animal hind feet, this site, must be avoided. 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 cause tissue damage. The injection is made into the pad or underside of the tail, not into the toes.

5. Inject pre-determined volume**
   Apply very gentle pressure to the plunger. The volume for injection is as per the animal ethics committee approved activity. The ink will disperse throughout the tissue. In UQBR the maximum volume injected is 1-2uL. The tattoo should be no wider than 2mm, too much pressure on the plunger at injection can result in the tattoo ink dispersing across the entire paw.

6. Immediately remove the needle, it is normal for a small amount of leakage to be visible.
   The injection site should be free of blood, ensure there are no cuts or scratches around the injection site. If you do see leaking ink gently wipe with a tissue, this will also avoid possible ingestion by the mother or littermates.

7. 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 excessively cleaning 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.

8. 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, or at a minimum replaced between cages.

9. 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.

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

Considerations for Neonates

- Consider using low volume syringes to improve volume accuracy when performing in neonates
  A 1 mL insulin syringe or standard 1mL syringe is ideal for this work, this 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.
- 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 INFORMATION

Table 1. Recommended values for Neonates using this technique

Values	Neonate
Needle Gauge	27-30 G
Needle Length	12 mm
Needle Depth at Injection	0.1 mm
Max Injection Volume	1-2 uL

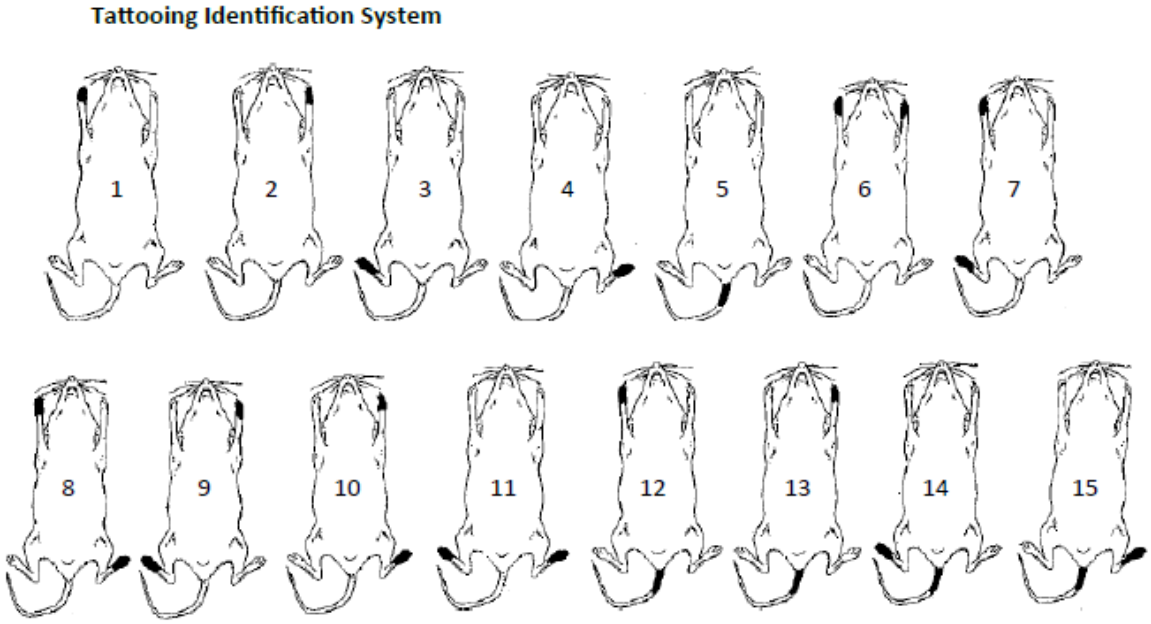

Figure 2 Tattoo identification system used across UQBR.

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.

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. UQ Biological Resources n.d., *UQBR SOP's, viewed 11 April 2019, https://biologicalresources.uq.edu.au/secure/reference-information#SOP's*
6. UQ Biological Resources, 2020 *UQBR Image Library*.