LAB_049 Injections - Intragastric Injections in Neonates

I. OBJECTIVE

To describe an appropriate, standard method of intragastric injection in neonatal mice.

II. COMMENTS / RECOMMENDATIONS

- Relative to animal ethics applications, when using this SOP, the following must be described in the individual ethics application: any experimental compounds or medications administered to neonates, the postnatal age of the pup, any intended variation to this SOP.
- Personal Protective Equipment (PPE) is facility and procedure dependent (e.g., handling potential zoonoses or carcinogens). Generally, PPE should include at least disposable gloves, long sleeved lab gown, face mask, safety glasses, hair bonnet, closed in shoes.
- Whenever performing procedures with neonatal pups (i.e. those still nursing), care is required to mitigate the risk of mis-mothering (i.e., dams may reject and cannibalise the pups once re-introduced). Avoid introducing foreign smells to the skin and hair of the pups by wearing a new set of gloves (for each litter), by avoiding spillage of chemicals or blood on the pup's skin, and by ensuring the workstation is clean (including between litters). Ensure that pups are all scent-marked with dirty bedding/ nesting material from the home cage immediately prior to their re-introduction. All pups should be handled in the same way and reintroduction should be performed *en masse,* never as one pup at a time. The period of separation should be as short as possible and any unnecessary disturbances should be avoided when monitoring the litter post-injection.

III. EQUIPMENT

- PPE, as required
- Ventilated workstation (e.g., change station or Bio-safety cabinet)
- Surface disinfectant
- Needle (30G) and syringe (≤1mL capacity) or an insulin syringe (30G)
- Substance for Injection
- Skin disinfectant (ethanol-soaked swab)
- Sharps Container
- "Home cage" containing the dam – standard housing boxes including sterile feed, water, appropriate nesting materials (to aid thermal support) and environmental enrichment.
- Temporary holding receptacle (to hold pups temporarily post injection) – clean, dry, and heated receptacles containing nesting material taken from the home cage (and a heat mat underneath).
- Fine tip permanent marker (optional for to identifying pups post injection)

IV. PREPARATION

1. Check the AEC approved protocol to ensure the correct procedures will be followed, and that the correct personnel will be doing the work
2. Prepare all equipment and the workstation
   - Turn on ventilated workstation (e.g., Aria change station or Biosafety Cabinet)
   - Use the disinfectant to clean contact surfaces prior to use

   Ensure the workstation and all equipment is clean and operating appropriately. Consider using clean bench coat if there is need to place the pup on the work surface.

Preparation of Injection Substance

1. Confirm the concentration and volume of the substance (as per the AEC approved protocol)
   Volumes may vary with age of neonates, but generally should not exceed 50uL. Relevant details should be clearly cited and justified in the AEC application.
   Temperature, pH, type and number of cells, hazardous substances (cytotoxic, radioactive, infectious), and highly viscous liquids must all be considered as they can influence animal welfare and scientific outcomes.
2. Ensure all needles and substances for injection are handled using aseptic technique.
   Aseptic technique will minimise the risk of contamination and associated complications (such as infectious disease and inflammatory reactions to foreign material).
3. Researchers have a responsibility to ensure they are aware of all relevant risks associated with compounds and materials to be used. This includes risks to human, animal, and environmental health. These risks and their management plans should be effectively communicated to all relevant personnel (e.g., animal technicians or other researchers working in the same space).
   If substances to be used are experimental or off-label (i.e. no Safety Data Sheet is available), the laboratory group may be required to manage these risks directly. This may require the research group to perform all animal care and husbandry (e.g., daily monitoring, changing cage bedding weekly, ensuring ad lib provision of food and water etc.)
4. Draw up the substances for injection so that the needle and syringe are ready for use.
   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 (for reference, see UQBR's Needle Use and Sharps Safety training).

V. PROCEDURE

Restraint for intragastric injection

Refer to LAB_006 Handling and Restraint in Mice and Neonates

1. Grasp loose skin behind the neck. For larger neonates the skin along the back may also be grasped.
   The pup should be gently immobilised. 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.

Restraint should, however, be secure. Excessive movement increases the risk of needle stick injuries or misplaced injection.

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.

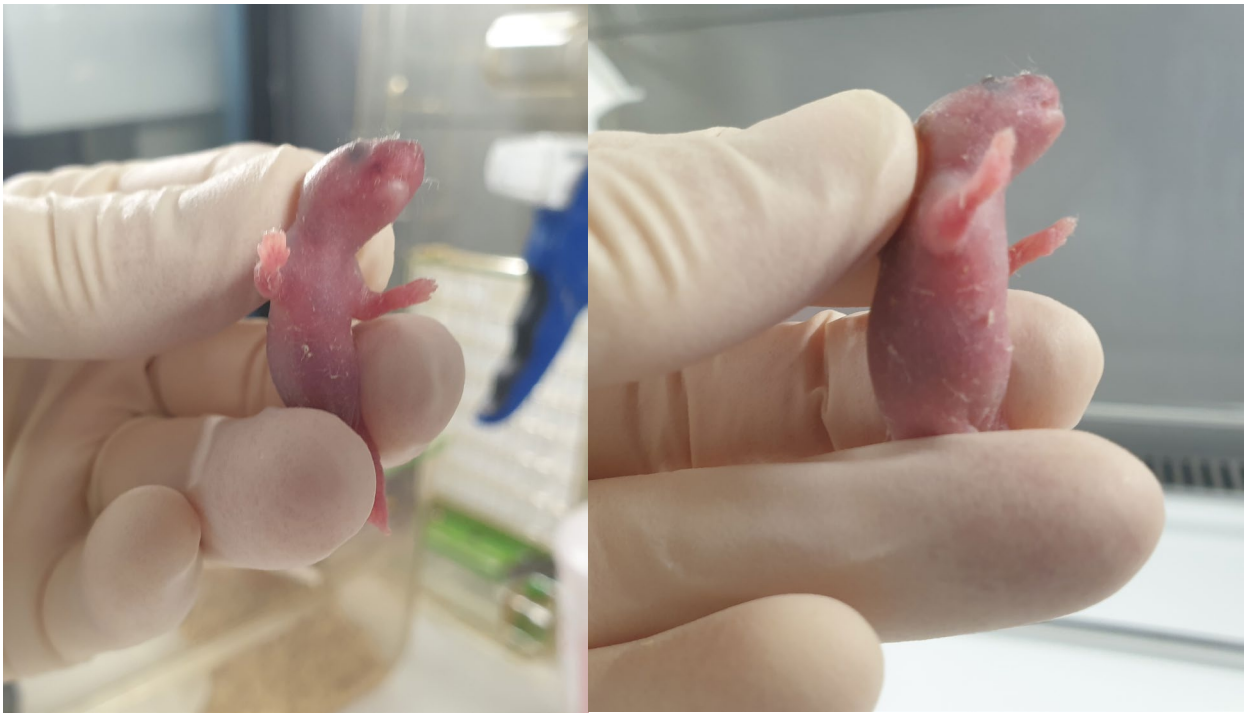

Figure 1. Restraint of neonate for injection (UQBR 2020).

Intragastric Injection Procedure

1. Identify the animal to be injected.
   Ensure the pup hasn't already been injected (see step 7).
2. Locate the milk spot in the stomach (usually visible in the upper left abdominal quadrant) and the liver (visible in the central umbilical quadrant) within the abdomen (see figure 2).
   Ensure the pup is not directly exposed to the air flow of the change station or biosafety cabinet as this can dry the skin and drop body temperature, making it harder to visualise the liver.
3. Ensure the pup is appropriately restrained
4. Holding the syringe in your dominant hand, insert the needle perpendicular to the skin to a depth of 2-5mm, so that the needle tip is within the stomach. The stomach is identified by the presence of a milk spot.

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 little resistance other than skin puncture, it is possible to go through the stomach resulting in a misplaced injection.

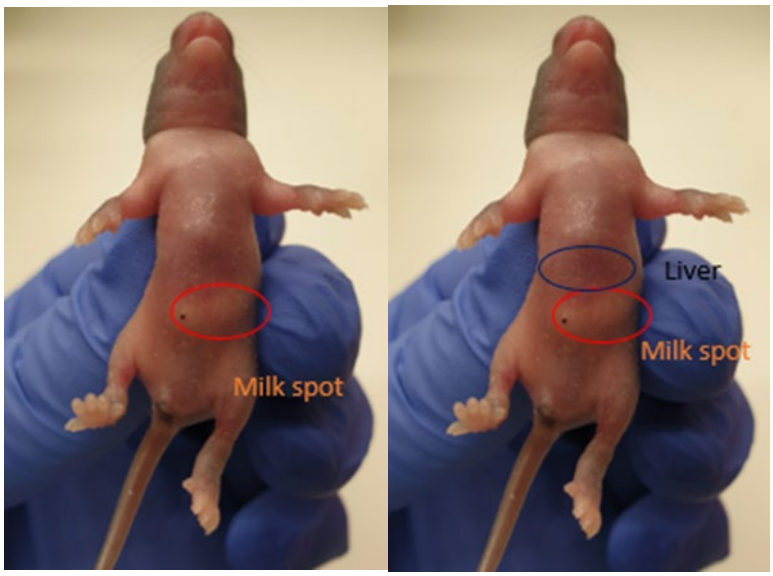

Figure 2. Location of milk spot (red circle) and the liver (blue circle). For intragastric injection the needle is inserted directly over the area of the milk spot (UQBR 2020).

5. Inject pre-determined volume into the stomach slowly.
   A maximum of 50uL should be injected, unless otherwise approved by the reviewing AEC.
   If there is leakage (e.g. > 5ul) of the substance immediately stop the injection and alter injection site.
6. Wait 3-5 seconds after the injection is complete, then slowly and smoothly remove the needle.
   There should be no cuts or scratches at the injection site. There should be no discharge of the injected substance or blood from the injection site. If there is any discharge clean the area, apply gentle pressure, and consider removing any excess residue (and odours) with a small amount of ethanol on a clean swab.
7. Cycle through all pups requiring injection, following a clear management strategy to avoid inadvertently injecting the same pup twice.
   For example, mark the injected pup with the fine tip permanent marker or place the injected pup into the temporary holding receptacle (before proceeding to the next pup).
8. Return all neonates to their home cage (with the dam) and continue to monitor them for up to 10 minutes.
   Returned pups must be continuously monitored until confident that the dam is appropriately attending to and nursing the pups. This should occur within 10 minutes of returning them to the home cage. If the mother is not attending to the pups within this time, or if cannibalism is observed intervention is required. If complications are observed during the reunion, communicate plans for intervention with your research group and animal facility staff.

9. Place the needle into sharps container and the syringe into the 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 the procedure, date and initials on cage card, as well as animal monitoring records.
    Injection procedures should also include the substance and volume injected. Records need to be clear and legible permitting others to read and understand.

VI. REFERENCES

1. NHMRC, 2008, *Australian code for the care and use of animals for scientific purposes*, National Health and Medical Research Council (NHMRC), available via: https://www.nhmrc.gov.au/about-us/publications/australian-code-care-and-use-animals-scientific-purposes
2. NHMRC, 2008, *Guidelines to promote the wellbeing of animals used for scientific purpose*, National Health and Medical Research Council (NHMRC), available via: 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	Approved Until
#2	MBS	30/11/2022	30/11/2025