LAB_043 Microinjection and electroporation of embryonic mice (Expiry: March 2026)

I. OBJECTIVE

To effect safe and humane experimental transfer of compounds into the developing embryonic mouse brain via surgical microinjection into the ventricular zones, with or without electroporation. Microinjection compounds usually contain experimental plasmid DNA, siRNAs, purified proteins, lipophilic dyes, fluorescent microspheres, inorganic microbeads, drugs, cells, and/or viruses.

II. COMMENTS / RECOMMENDATIONS

- Users must keep monitoring records, which includes surgical records (example templates can be obtained by contacting the UQBR Veterinarians or Animal Ethics Unit Veterinary Officer).
- Any associated experimental compounds or medications (including your anaesthetic protocol) must be detailed within the Animal Ethics Committee (AEC) application.
- PPE is facility dependent, however, this should at least include disposable gloves, long sleeved lab gown, face mask, safety glasses, hair bonnet, closed in shoes.
- Wherever possible, active heating (e.g. a heat mat) must be used at all times.
- Clean surgical technique must be practiced, as per LAB_002 Clean Technique for Laboratory Animal Surgery
- Wherever practicable, aseptic surgical technique must be practiced, as per LAB_001 Aseptic Technique for Laboratory Animal Surgery
- In the event of equipment failure, or anaesthetic recovery mid-surgery, "alleviating unanticipated pain and distress must take precedence over an individual animal reaching the planned endpoint of the project, or the continuation or completion of the project. If necessary, animals must be humanely killed without delay" (Clause 2.4.18, Australian code for the care and use of animals for scientific purposes 8th Edn., 2013)
- The time from induction of anaesthesia to the completion of surgery **must not exceed 30 minutes,** as longer surgeries negatively impact litter viability. This may prevent investigators from being able to inject and electroporate entire litters in utero, especially when using mice with large litter sizes (e.g. Swiss, CD1).

III. EQUIPMENT

- Disinfectants: surface disinfectant (e.g. 70% ethanol) and skin disinfectants (e.g. chlorhexidine based). Refer to LAB_001 Aseptic Technique for Laboratory Animal Surgery and LAB_002 Clean Technique for Laboratory Animal Surgery for options.
- Clean recovery boxes – standard housing boxes including sterile feed, water, appropriate nesting materials (to aid thermal support) and environmental enrichment.
- Active heating equipment (e.g. fit for purpose heat mats, Bair-hugger device, Aria Ventilated Cabinets®)
- Anaesthetic agents – as per AEC approved protocol
- Analgesic agents – as per AEC approved protocol
- Experimental compounds for injection +/- sterile non-toxic dye for injection (e.g. Fast Green dye at 0.0125 - 0.05% in sterile saline) – as per AEC approved protocol
- Ophthalmic lubricant (non-medicated, viscous and pH neutral: e.g. Refresh "Lacri-lube"©, Visco-tears© gel)
- Electric clippers or depilatory cream (e.g. Nair hair removal cream©)
- Sterile surgical instruments
  - Including: scalpel, fine surgical scissors, ring forceps (size appropriate to the pups' gestational age), fine forceps, haemostats, needle drivers
- Sterile surgical consumable
  - Including: gauze, cotton tips, absorbable suture (size: 5-0 or 6-0), warmed normal (0.9%) saline (sterile), 7mm or 9mm wound clips and wound clip applicator.

- Micro-injection apparatus
  - sterile pulled glass pipette attached to a micropipette
- Microelectrodes
- Electroporation generator

IV. PROCEDURE

1. Prepare yourself and the work station as per LAB_001 Aseptic Technique for Laboratory Animal Surgery / LAB_002 Clean Technique for Laboratory Animal Surgery
2. Prepare clean, warm recovery boxes (e.g. resting on a heat mat).
3. Anaesthetise the animal, as per AEC approved protocol.
4. Apply ophthalmic lubricant to both eyes, using a sterile cotton tip.
5. Prepare the animal for surgery in dorsal recumbence, as per LAB_001 Aseptic Technique for Laboratory Animal Surgery / LAB_002 Clean Technique for Laboratory Animal Surgery
6. Check for the absence of a withdrawal reflex. If a withdrawal reflex is present, the animal is not sufficiently anaesthetised and anaesthetic depth needs to be increased prior to proceeding.

   If movement of skeletal muscle, or withdrawal reflexes are present at any point throughout the procedure, activity must stop and only resume once sufficient anaesthetic depth regained. If you are having difficulty maintaining appropriate anaesthetic depth consult a UQBR veterinarian (once the animal has recovered, and before proceeding to anesthetise any more animals).
7. Perform a ventral mid-line laparotomy (through the linea alba) using either scalpel or fine surgical scissors. The incision should be no larger than required to exteriorise the gravid uterine horns (~1.5-2cm incision length).

   Laparotomy must be performed in two stages: skin, followed by the muscular abdominal wall.
8. Place sterile gauze moistened with normal saline around the incision. Identify and then gently exteriorise the uterine horns, resting them onto the moist gauze. Once exteriorised, sterile saline must be applied to the exposed tissue every 1-2 minutes, until it is replaced for wound closure. This is essential to prevent serosa desiccation, and associated necrosis.

   Note: Particular care is required when handling the gravid reproductive tract; ligaments, blood vessels and associated soft tissue structures can easily be damaged if there is any undue force applied.
9. Embryos (or pups) are then gently positioned using ring forceps and/or gloved fingers and the brain of each embryo is then microinjected using a glass microinjection pipette with the desired injectate solution mixed with non-toxic dye, as required to visualize the injectate. The volume of injectate is less than or equal to the volume of the CSF at any given developmental stage, and is approximated by the extent of dye immediately following microinjection.

   If the pressure used to hold individual embryos for this step is excessive the amniotic sac will rupture when the micropipette is inserted (like popping a balloon with a pin). For this reason, ensure embryos are held securely but with as little force as necessary.
10. If electroporation is required to incorporate charged substances such as DNA into a specific tissue region, microelectrodes are placed over the tissue region of interest and 10-80V square wave 50ms pulses (depending on the developmental stage) are administered 3-10 times using an electroporation generator.

    Model specific consideration for electrode placement is required given variable effects associated with differences in direction of charge transfer.
11. The uterus is then gently replaced into the abdomen.

12. The muscular abdominal wall must be closed using absorbable sutures. The skin must be closed separately using either suture material or wound clips. The surgical site is then gently cleaned with gauze or a cotton tip moistened with skin disinfectant to remove any blood contamination.
13. Place the animal into a recovery box, maintained on a heat mat until fully ambulatory. If available, recovery boxes may then be placed into a climate controlled, Ventilated Cabinets® for ~12 hours recovery.
14. Clean and disinfect all equipment between each animal.
15. Continuously monitor all mice during surgery and throughout the recovery phase until fully ambulatory. Mice should be reassessed within 6 hours post recovery, then at least daily for the following 2 days. Ongoing monitoring is as described by the approved AEC activity.

V. BIBLIOGRAPHY

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