University Animal Care Committee Standard Operating Procedure		
Document No: 7.26	**Subject:** Intrafemoral Injections (Mice)	
Date Issued: September 19th, 2019	**Revision:** 1	**Page No:** 1

Location: Queen's University

Responsibility: Principal Investigators, Research Staff, Veterinary Staff

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe the standard method used for intrafemoral injections in mice.

1. **Introduction and Definitions:** This technique allows cells to be injected into the bone marrow without repeated needle entry. This method is used primarily for cell engraftment purposes. The maximum injection volume must not exceed 30ul. Only one leg is to be used per mouse, with a maximum of two attempts.

Abbreviations: Animal Care Services **ACS**, Principal Investigator **PI,** subcutaneous **SC**, intravenous **IV**, intraperitoneal **IP,** intramuscular **IM**, per os **PO,** per rectum **PR**

2. **Materials:**
   - Hair clippers
   - Chlorohexidine scrub
   - 70% alcohol
   - Iodine
   - Eye lubricant and sterile swab
   - 25g needle
   - Hamilton syringe
   - Sterile 2" x 2" gauze
   - Surgical tape
   - Heat disk/lamp
   - Anaesthetic vaporiser and isoflurane (or other anaesthetic as approved in the protocol)
   - Analgesic
   - Tissue glue
   - Recovery cage lined with paper towel
   - Food grade dye (used to practice the technique and confirm successful implantation)

3. Procedures:

- Aseptic technique must be followed.
- Set up work surfaces and preload syringe with cells, advancing the cells to the tip of the 25g needle to reduce dead space.
- Anesthetize the mouse in an induction chamber, and transfer to nose cone as per SOP #7.6 “Anaesthesia in Mice”.
- Lay the mouse in dorsal recumbency and shave the patella.
- Wash the site with chlorhexidine scrub.
- Don surgical gloves, and disinfect the skin as per SOP #7.3 “Aseptic Surgical Techniques (Mice)”.
- Cut a hole in the centre of a sterile 2’x 2’ gauze and drape it over the knee.
- A keyhole in the skin can be made lateral to the patella for ease of visualization.
- To isolate the right knee, use the left thumb and forefinger to stabilize the femur (figure 1).
- Using the right ring finger, apply gentle pressure to the tibia to flex the knee (figure 2).
- Position the leg so the femur is perpendicular to the table surface (figure 2).
- Surgical tape may be used to help position the mouse.
- Grasp the 25 gauge needle with the right thumb and forefinger, and locate the patellar tendon.
- Insert the 25 gauge needle through the patellar tendon, between the condyles and advance the needle 2-4mm in a twisting motion through the bone (figure 3).
- Confirm placement by carefully moving the needle and observing movement of the entire leg.
- With the 30cc Hamilton syringe, feed the needle into the 25 gauge needle and slowly inject the desired amount of cells. Remove the 25 gauge needle in the same twisting motion.
- Apply tissue glue to close the keyhole incision.
- Move the animal to the recovery cage, and administer analgesia as per the approved animal use protocol.
- Transfer the animal back to the home cage once ambulatory.
- Monitor the animal for signs of pain, bleeding, and infection. Contact the veterinary team if animal displays signs of distress such as irregular gait, changes in weight (15% weight loss), ruffled fur, hunched back, dehydration, decreased activity, vocalization of stress.

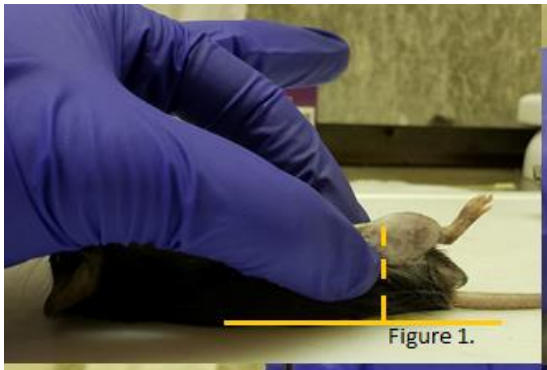

Figure 1.

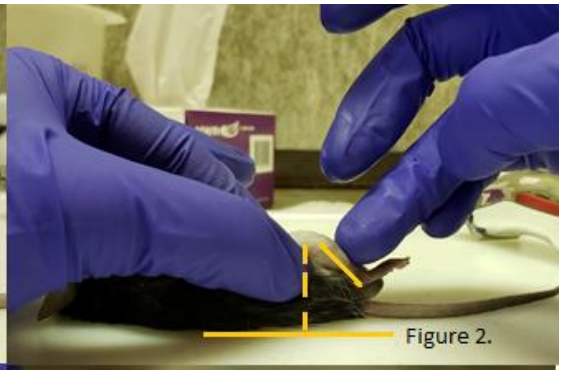

Figure 2.

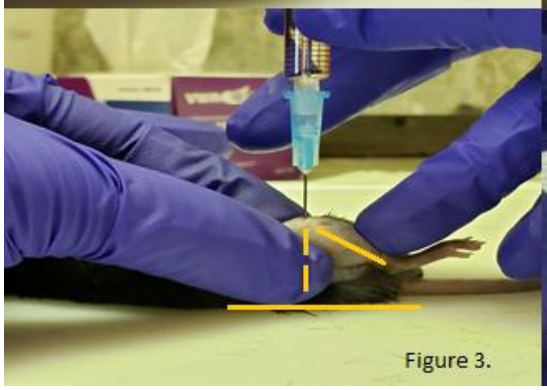

Figure 3.

Figure 4. Presence of dye in the bone marrow confirms successful injection (blue)

References:

SOP Revision History:

Date	New Version
10/31/2022	Triennial review. Updated format and procedure.