University Animal Care Committee Standard Operating Procedure		
Document No: 7.9	**Subject:** Injection Techniques in Mice	
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Location: Queen's University

Responsibility: Principal Investigators (PI), Research Staff, Veterinary Staff

Purpose: The purpose of the Standard Operating Procedure (SOP) is to describe common injection techniques used for mice.

- **Introduction and Definitions:**

The injection methods described within an Animal Use Protocol (AUP) must be followed at all times. The following guidelines provide recommended injection sites, needle sizes and maximum dose volumes. "Good Practices" include:

- All animals securely and safely restrained prior to injecting.
- Only three attempts per site should be practiced. If unsuccessful, allow another (trained and competent) person to collect the sample.
- Use the appropriate gauge needle and volume for the injection site based on the size of the mouse.
- Before injecting any substance, aspirate first to ensure appropriate placement of the needle (excluding intravenous injections).
- Always inject with the bevel up on the needle.
- Always ensure the substances you are injecting are sterile, and use sterile technique.
- Each and every animal requires a new sterile syringe and a new sterile needle. With small volumes, it is preferable to dilute the injectable agent to a 50% or less solution to ensure accurate dosing.
- Disinfecting the skin with alcohol is recommended but not mandatory for subcutaneous injections.
- Disinfecting the skin with alcohol <u>is mandatory</u> for intravenous, intraperitoneal, intramuscular, intradermal, and all biohazardous injections.
- Choose the appropriate administration route for the substance to be injected.

Recommended Needle Sizes and Volumes

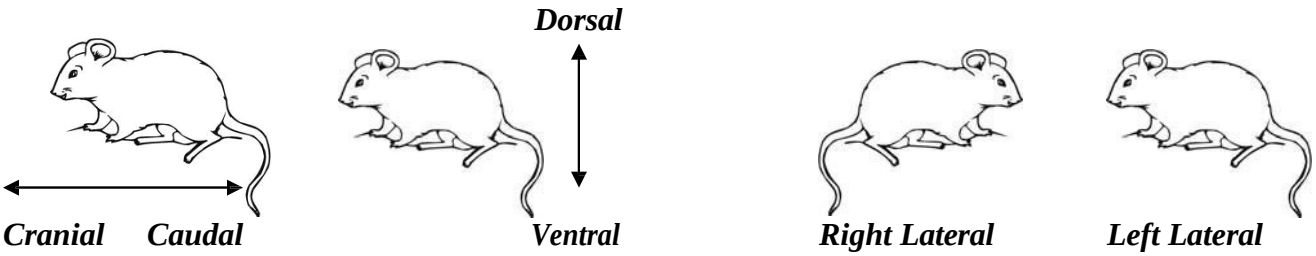
Length of needle: ½ to ¾ inch

	Intradermal ID	**Subcutaneous SC**	**Intramuscular IM**	**Intraperitoneal IP**	**Intravenous IV**
Recommended Gauge (maximum)	27 (26)	26 (25)	26 (25)	26 (25)	26 (25)
Good Practice Volume (Max per site)	0.05 ml	1 ml (2ml)	0.05 ml	1 ml (2ml)	0.2 ml

- **Materials**
  - Restrainers
  - Sterile syringes
  - Sterile needles (multiple sizes ranging from 25-30g)
  - 70% alcohol swabs
  - Clippers
  - Injectable solution
  - Anaesthetics as required
  - Isotonic fluids such as Lactated Ringers, 0.9% NaCl

2. Procedures

Anatomical Terms of Location



Intradermal Injections (ID)

- Mice may need to be sedated.
- Each and every animal requires a new sterile syringe and a new sterile needle.
- Load the syringe and needle with appropriate volume to be injected.
- Safely restrain the animal on the table.
- The hair may need to be shaved to allow for better view.
- Disinfect the injection site with 70% alcohol.
- Pinch the skin upward or lay the syringe with bevel facing upward along the side of the animal's body.
- Insert the needle just burying the bevel. Pivot the needle gently to create small pocket.
- Inject slowly and look for a bleb to form within the skin layer (should feel a slight resistance).
- If the skin doesn't appear to rise immediately, the substance is going deeper than the intradermal layer. Stop the injection, remove needle and reposition.

Subcutaneous injection (SC)

- Each and every animal requires a new sterile syringe and a new sterile needle.
- Load the syringe and needle with appropriate volume to be injected.
- Safely restrain the animal.
- The most common injection site is the loose skin around the neck and shoulder area.

Grasp the scruff and tent the skin upward. Other SC sites include the dorsolateral thorax and flank, depending on the volume to be administered.

- Palpate toward the bottom of the tented skin to ensure interstitial space is accessible.
- Disinfect that injection site with 70% alcohol (if required).
- Insert the needle (bevel up) into the base of the tented region.
- Aspirate to ensure the placement of the needle is correct. Proper placement should yield an air bubble and no aspirate in the hub of the needle. If any fluids are seen; stop, reload with new syringe and needle, check injection site for trauma, reposition needle and attempt again.
- After ensuring proper placement, inject.
- If resistance is felt during the injection, stop and (slightly) reposition the needle.
- When injecting larger volumes or viscous substances, use the largest acceptable gauge needle for the animal.

Intramuscular Injections (IM)

- Each and every animal requires a new sterile syringe and a new sterile needle.
- Load the syringe and needle with appropriate volume to be injected.
- Safely restrain the animal using either physical restraint or anesthesia.
- Place your hand on the inside of the animal's hind leg and gently extend the leg, stabilizing the muscle.
- Palpate the hamstrings on the caudal aspect of the femur.
- Disinfect the injection site with 70% alcohol.
- Care must be taken to avoid injecting material near the sciatic nerve which runs superficially along the caudal aspect of the femur in the thigh. The needle must be directed caudally with the bevel up.
- Aspirate to ensure the placement of the needle is correct. Proper placement should yield an air bubble and no aspirate in the hub of the needle. If any fluids are seen; stop, reload with new syringe and needle, check injection site for trauma, reposition needle and attempt again.
- After ensuring proper placement, inject.

Intraperitoneal Injection (IP)

- Each and every animal requires a new sterile syringe and a new sterile needle.
- Load the syringe and needle with the appropriate volume to be injected.
- Safely restrain the animal using the scruff technique and place in dorsal recumbency with the head tilted slightly downward to provide a clear view of the abdomen.
- Identify the midline of the abdomen. The optimal side to inject IP in a mouse is their right side, avoiding the cecum which typically lies on the animal's left side.
- Disinfect the injection site with 70% alcohol.
- The needle should be inserted bevel up in line with the natural extension of the hip, between the two lower nipples and angled at ~45 degrees to the abdomen. The angle must be sharp enough to penetrate into the abdominal cavity, affirming the site is not subcutaneous.

- Aspirate to ensure the placement of the needle is correct. Proper placement should yield an air bubble and no aspirate in the hub of the needle. If any fluids are seen, stop and return the mouse to its home cage. If the mouse shows no sign of discomfort restrain again, check injection site for trauma, reposition needle, ensure proper placement within the cavity and re-attempt. Best practice is to draw up another non-contaminated injection (with a new sterile needle and syringe), however if this is not possible the needle must be substituted for a new one. If the mouse is in a hunched position or you can see shadows (indicative of a hematoma) within the peritoneal cavity, the mouse needs to be euthanized immediately as the inferior vena cava may have been punctured.