1. **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.

Techniques which are considered to be good practice are:

- All animals must be 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 rat.
- Before injecting any substance, aspirate first to ensure appropriate placement of the needle.
- 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.
- Choose the appropriate administration route for the substance to be injected.

### Recommended Needle Sizes and Volumes

*Length of needle: 5/8 or 3/4 inch*

<table>
<thead>
<tr>
<th>Injection Type</th>
<th>Intradermal (ID)</th>
<th>Subcutaneous (SC)</th>
<th>Intramuscular (IM)</th>
<th>Intraperitoneal (IP)</th>
<th>Intravenous (IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recommended Gauge (maximum)</strong></td>
<td>27 (25)</td>
<td>26 (21)</td>
<td>26 (25)</td>
<td>26 (23)</td>
<td>26 (23)</td>
</tr>
<tr>
<td><strong>Good Practice Volume (maximum)</strong></td>
<td>0.1 ml</td>
<td>5 ml (10ml)</td>
<td>0.1 ml</td>
<td>1 ml (10ml)</td>
<td>0.5 ml (1ml)</td>
</tr>
</tbody>
</table>
2. Materials:
- Restrainers and drapes as required
- Sterile syringes
- Sterile needles (multiple sizes ranging from 21-27g)
- 70% alcohol swabs
- Clippers
- Injectable solution
- Anaesthetics as required

2. Procedures:

Anatomical Terms of Location

Intradermal Injections (ID)
- 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 a better view.
- Disinfect the injection site with 70% alcohol (if practical).
- Pinch the skin upward or lay the syringe with bevel facing upward along the side of the animals body.
- Insert the needle just burying the bevel. Swivel needle to create small pocket.
- Inject slowly and look for a dome shape 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 on the table.
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 exposed.
- 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. The use of a butterfly catheter may facilitate the procedure.

**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 or chemical restraint.
- 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 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 rat is their right side because the rat has a very large cecum which usually lies on the animal’s left side.
- Disinfect the 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.
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.

**Alternative Intraperitoneal technique:** This hold was developed for animals that are accustomed to handling. It alleviates stress to the animal, and allows for clear visualization of the needle hub.

![Image of rats being injected](image-url)