1 PURPOSE

This SOP describes the methods and considerations for the safe injection of rodents used for research at Carleton University.

2 CONSIDERATIONS

2.1 Based on the type of injection, there will be different absorption times. From fastest to slowest these are: intravenous, intraperitoneal, intramuscular, subcutaneous, and intradermal

   2.1.1 Different substances will have a recommended route of administration, contact the supplier if unclear

2.2 The use of restraining aids or an assistant to restrain animals during injections

2.3 Substances should be a neutral pH and warmed whenever possible

2.4 All students must be trained on proper injection technique by Animal Care and Veterinary Services before injecting any live animals

3 GENERAL PROCEDURE

3.1 Collect all supplies before beginning any procedure

   3.1.1 Syringes should be loaded before animals are removed from their cages

   3.1.2 Any vials or syringes not used immediately should be labeled with the substance, concentration, and date

   3.1.3 Some substances are light sensitive, care should be taken to store these in brown vials or covered

   3.1.4 When drawing up multiple doses from a single vial, the top should be swabbed with alcohol between each use unless the substance may be affected by alcohol

   3.1.5 Needles and syringes should be left capped on a stable surface until use

3.2 Check syringes for bubbles or blunted needles, remove bubbles and replace blunted needles

3.3 Never inject an animal if you are unsure of the contents of a needle

3.4 Restrain the animal appropriately for the technique (SOP EXP-02 Rodent Handling and Restraint)

3.5 Insert the needle, keeping the bevel of the needle up and inject in a smooth motion without stopping

3.6 Replace the animal in its home cage and monitor for any bleeding

3.7 Record the procedure on the cage card or in a logbook available in the animal room

3.8 Record should include date, substance injected, volume injected, and route, and be initialed by the person who performed the procedure

3.9 If complications occur, contact Animal Care and Veterinary Services immediately so that a health record may be created and the animal monitored
4 INTRAVENOUS INJECTION

4.1 In mice and rats, intravenous (IV) injections are delivered via the lateral tail veins.

4.2 It is recommended that rats be anesthetized for tail vein injections, mice may be restrained in an appropriate restrainer.

4.3 To make the vein more visible, apply heat from a heating pad, heat lamp, or warm water. Rubbing the tail with an alcohol swap can also make the vein stand out more.

<table>
<thead>
<tr>
<th>Species</th>
<th>Needle size</th>
<th>Maximum volume to inject</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>27-30 gauge</td>
<td>0.2ml</td>
</tr>
<tr>
<td>Rat</td>
<td>25-27 gauge</td>
<td>0.5ml</td>
</tr>
</tbody>
</table>

4.4 Isolate the tail vein, swab the area with alcohol, and inject at a shallow angle (almost parallel) with the bevel of the needle up.

4.4.1 Start closer to the tip of the tail.

4.5 Aspirate the needle by pulling gently back on the plunger. A small flash of blood should appear. If no blood appears, remove the needle and try again (a new needle may be required).

4.5.1 Caution must be used as aspirating too hard will collapse the tail vein.

4.6 Slowly inject the substance.

4.6.1 If you feel resistance it is likely that the needle has left the vein. Stop, reposition, and aspirate before continuing.

4.6.2 The blood in the vein will be displaced with proper injection technique, resulting in a “blanching” of the tail.

4.7 When finished, remove the needle and apply pressure to the tail until bleeding stop.

4.8 Discard the needle into a sharps container without recapping.

4.9 Place the animal back into its cage and observe for any complications.

4.10 Document injection on cage card or in animal records, maintained in the holding room.

4.11 Complications

4.11.1 Tail necrosis: Perivascular (outside of the vein) injection of fluids can potentially lead to necrosis of the tissues surrounding the injection site.

4.11.2 Hyperthermic signs include: Red extremities, rapid respiration or panting, decreased activity.
5.1 In mice and rats, intraperitoneal (IP) injections a substance into the peritoneal cavity

<table>
<thead>
<tr>
<th>Species</th>
<th>Needle size</th>
<th>Maximum volume to inject</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>23-27 gauge</td>
<td>2-3ml</td>
</tr>
<tr>
<td>Rat</td>
<td>22-25 gauge</td>
<td>5-10ml</td>
</tr>
</tbody>
</table>

5.2 Due to the position of the many organs within this area, proper care and technique is necessary to prevent injury to the animal. In addition, substances must be of neutral pH and non-irritating.

5.3 To landmark the area, draw a line down the midline and across the umbilicus to divide the abdomen into quadrants. Either lower quadrant may be used to inject with the needle inserted towards the head of the animal.

5.3.1 If doing multiple injections on the same animal, alternate sides between injections

5.4 Landmark the area to inject and insert the needle on a 30-45° angle with the bevel up.

5.5 Aspirate the needle by gently pulling back on the plunger.

5.5.1 Do not inject if any material is drawn into the syringe (ex. Blood, bile, urine)

5.5.2 If this occurs please the mouse back in it’s cage and notify ACVS for monitoring

5.6 Inject the substance at a steady rate.

5.7 Withdraw the needle and discard into sharps container without recapping.

5.8 Replace the animal in the cage and monitor for bleeding.

5.9 Document the procedure on the cage card or in animal records.

5.10 **Mouse IP Injections**

5.10.1 Restrain the mouse by scruffing (see SOP: Rodent Handling and Restraint). Tilt the mouse with its head slightly toward the ground (head lower than the hind legs). This allows the abdominal viscera to shift cranially, minimizing the accidental puncture of abdominal organs at the injection site.

5.10.2 Place the mouse back in the cage and monitor for any complications.

5.11 **Rat IP Injections**

5.11.1 Restrain rat using one or two persons restraint method (see SOP: Rodent Handling and Restraint)

5.11.1.1 It is recommended to use a two person method to give an IP injection to a rat

5.11.2 Landmark and inject as listed in 5.4.2 to 5.4.6 above.
5.12 **Complications**

5.12.1 Bleeding at injection site – apply pressure until the bleeding stops and clean with gauze and water

5.12.2 Peritonitis – inflammation or infection of the peritoneal cavity

5.12.3 Paralytic ileus due to substance injected

5.12.4 Laceration of abdominal organs

5.12.5 Injection into the gastrointestinal tract or bladder

6 **SUBCUTANEOUS INJECTION**

6.1 The most common location for subcutaneous (SC or SQ) injections in mice is the scruff or over the flank. In rats the most common site is over the flank.

6.2 If an animal has had surgery the incision site should be considered before giving a SC injection (ie. a SC injection cannot be given in the scruff of an animal who has had a mini-pump implanted.)

<table>
<thead>
<tr>
<th>Species</th>
<th>Needle size</th>
<th>Maximum volume to inject</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>25-27 gauge</td>
<td>2-3ml</td>
</tr>
<tr>
<td>Rat</td>
<td>22-25 gauge</td>
<td>5-10ml</td>
</tr>
</tbody>
</table>

6.3 Restrain the animal

6.3.1 For mice either scruff and place on wire bar hopper or use a towel to prevent movement

6.3.2 For rats a towel wrap to limit movement; can be used for both the scruff and flank.

6.4 Holding the syringe in your dominant hand, and with the bevel of the needle facing upwards, insert the needle through the skin at the base of this tent.

6.5 Aspirate the needle by pulling back gently on the syringe

6.5.1 Do not continue if blood or air is seen

6.5.2 If air is drawn up, the needle has gone through the skin again. Remove the needle, eject the air bubble, and begin again

6.6 Inject the substance

6.7 Withdraw the needle and discard into a sharps container without recapping

6.8 It is common to see surface bleeding after a SC injection. Clean the area and monitor. If bleeding persists contact ACVS

6.9 Place the animal back into its cage and observe for any complications. Replace the mouse in the cage

6.10 Document injection on cage card or in animal records, maintained in the holding room.

6.11 **Complications**

6.11.1 Bleeding at injection site – apply pressure until the bleeding stops and clean with gauze and water
6.11.2 Inflammation, abscesses or ulcers at the injection site due to pH of solution, contamination or tissue irritation

7 SAFETY

7.1 In the event of an injury seek medical attention (x4444) and notify your supervisor. In the case of an animal bite refer to SOP: Animal Related Injury. In case of a needle stick injury refer to SOP OHS-02 Animal Related Injury

8 REFERENCES

SOP ANA-03 Mouse Anesthesia

SOP ANA-04 Rat Anesthesia

SOP EXP-02 Rodent Handling and Restraint

SOP OHS-02 Animal Related Injury


