UBC ANIMAL CARE COMMITTEE
TECH 11b - Subcutaneous Injections in Adult Rats SOP

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PURPOSE:

- To describe the procedure for administering subcutaneous (SC, SQ) injections in adult rats.
- This Standard Operating Procedure (SOP) follows the Canadian Council on Animal Care (CCAC) current guidelines for acceptable injection volumes and sites in rodents.

RESPONSIBILITY:

- Those trained persons listed on an approved Animal Care Committee (ACC) Animal Care Protocol who are responsible for performing subcutaneous injections.
- All animal users performing subcutaneous injections in rodents must have successfully completed the UBC Animal Care Services (or equivalent) Introduction to Working with Rodents in Research (IWRR), Rodent Restraint/SQ/IP injections (RSCIP), and Rodent Anesthesia (RA; if applicable) courses.

MATERIALS: (*can be purchased from Animal Care Services*)

- Sterile syringes (typically 3-5 ml)
- Sterile needles (23-25G; 5/8" length)
- Sterile substance to be injected (in sterile, multi-dose vial)
- 70% isopropyl alcohol
- 2" x 2" Gauze
- Sharps container
- Weigh Scale
- Heat source to warm substances to be injected
- Optional: towel for wrapping rats or restrainer

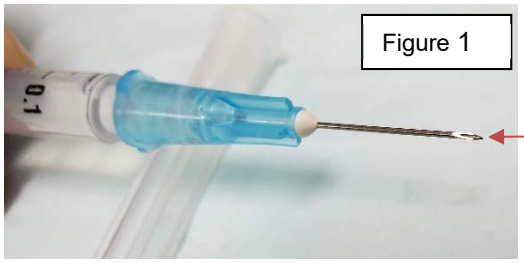
Table 1 - RECOMMENDED NEEDLE SIZE AND MAXIMUM VOLUME OF ADMINISTRATION

Species	Needle Gauge and Length	Maximum Volume Per Site for SQ Injection for Drug Administration*	Maximum Total Volume for Drug Administration*
Rat	23-25G; 5/8" length	5 ml/kg per site	10 ml/kg
		Maximum Volume Per Site for SQ Injection for Fluid Therapy**	**Maximum Total Volume for Fluid Therapy****
		20 ml/kg per site***	Up to 40 ml/kg***

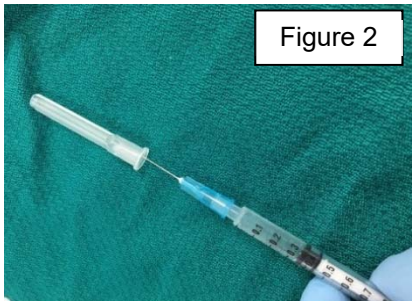
*Greater than the recommended volumes of drugs should not be given unless justified and approved on the Animal Care Protocol and increased monitoring for complications implemented. Volume does not include Complete Freund's adjuvant (CFA) administration which should be less (0.1ml CFA/site).
**For animals that are dehydrated, unwell or pre/post op, up to 20ml/kg per site of replacement fluids (0.9% saline or LRS) may be acceptable. See UBC ACC Guidelines and SOP for the Maintenance of Fluid Homeostasis in Animals.
*** for larger volumes, inject over multiple sites, up to a maximum of 4 sites (2-3 sites is preferred)

PROCEDURE:

1. Warm up the container of substance to be administered.
    a. E.g. heating pad, water bath, or holding vial/prepared syringe in hand to warm up.
    b. For small volumes, holding the prepared, filled syringe in hand for 10 seconds is sufficient.
    c. Do not overheat beyond 37°C.
2. Weigh the animal and calculate the volume to be administered (refer to Table 1 for maximum recommended volume and below for how to calculate volume).
3. Disinfect the top of the container with alcohol-moistened gauze. Allow to dry.
4. Safely uncap the needle. Maintain sterility of the needle.
5. Draw up the amount of pre-warmed solution to be administered into the syringe and needle.
    a. It is helpful to turn the needle so that the bevel points "up", and turn the syringe so the numbers on the syringe barrel can be read (see Figure 1).



6. The needle can be slid back into the cap loosely without handling either the cap or the needle (see Figures 2 and 3 below) while you restrain the animal.



7. Place rat on a stable surface.
8. Restrain the rat appropriately, with or without a cloth or small towel to cover the face.
    a. Towel is used to cover the rat's face so it cannot see and keep it calm.
    b. The weave of the towel should allow the rat to breathe once wrapped and not be so loose a weave that the rat can catch and tear its toenails.
    c. For calm rats used to handling and injections, injecting them while gently held against the side of the cage or while on your lab is possible.
9. With your non-dominant hand over the rat's back, wrap fingers around its body and tuck it in close to you so it cannot move (forward or backward) while the injection is being given.
    a. Use only enough pressure to keep the rat in place, ensuring it can breathe easily.
10. Tent (lift) the loose skin with the same hand (see Figures 4 & 5).
    a. If injecting substances that would be harmful if injected into yourself (i.e. tumour cells, bacteria, etc.), it is advised to use a restrainer (see Figure 6) or anesthetize the rat. Use padded forceps to grasp and lift the skin if injecting hazardous substances.

Figure 4

Restraining without towel on a table top

Figure 5

Restraining with a towel on a table top

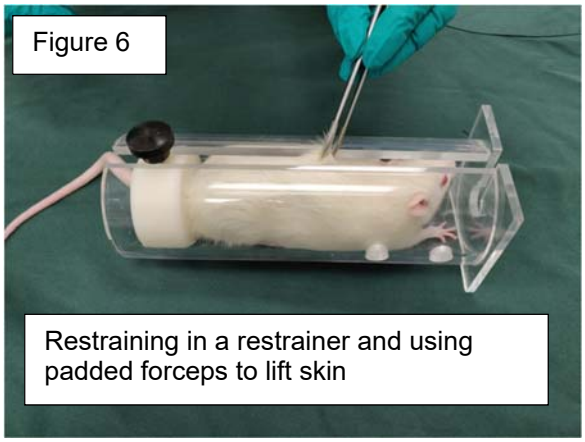


Figure 6

Restraining in a restrainer and using padded forceps to lift skin

11. Insert the needle into the base of the tented skin with your dominant hand.

a. Insert the full length of the needle, parallel to the body, with the bevel facing up (see Figure 7).

Figure 7

12. Pull back on the plunger to ensure negative pressure and that nothing is aspirated (air or blood) before injecting. Do not allow the needle to move further in or out of the skin while pulling back on the plunger or injecting.

a. If blood is drawn back into the hub of the needle, you have hit a capillary. Withdraw and re-attempt with a new needle, new syringe and fresh solution.

b. If air is drawn back into the syringe, you have exited the skin. Pull the needle out of the skin, expel the air, then re-attempt with the same needle. Change needle after 2 attempts. A total of 3 attempts is permitted.

13. If nothing enters the syringe (negative pressure), proceed with the injection - depress the plunger until the solution has been fully administered. Withdraw the needle from the skin.
    a. See suggestions at the end of this document (Appendix 1) on holding the syringe to pull back and inject, without readjusting your hold on the syringe.
14. Discard syringe and needle directly into a sharps container.
    a. DO NOT RECAP once it has been in an animal.
15. Return the animal to its cage and observe for any complications (see below).
16. Note procedure (drug, dose, route, volume, and any complications) on cage card/monitoring records.

CALCULATING VOLUME (IN ML) TO BE ADMINISTERED:

- Convert animal's weight from grams to kilograms
    - Divide the weight in grams by 1000
    - E.g. 250g rat ÷ 1000 = 0.25kg
- Calculate the volume to give in ml
    - = volume (ml/kg) x weight of animal (kg)
    - E.g. For a 250g rat getting 10 ml/kg
      Volume (ml) = (10 ml/kg x 0.25 kg) = **2.5 ml**

IMPORTANT NOTES:

- Use the manufacturer's recommended route of injection or contact your Facility Veterinarian for further guidance. Some drugs may have adverse side effects or cause discomfort if injected via a non-recommended route.
- A new sterile syringe and needle must be used for each animal.
- The volume to be injected should be the smallest volume possible for drug administration and not exceed the current recommended volume guidelines unless justified and approved on the Animal Care Protocol (see Table 1 above).
- Follow the recommended needle size (see Table 1 above). Using a larger than recommended needle size must be approved on the Animal Care Protocol (i.e. if injecting viscous liquids).
- All substances for injection must be sterile since contamination can cause infection and/or irritation at the site of injection.
- All substances for injection must be biocompatible since foreign body reactions may lead to irritation, infection and lesions at the injection site.
- Warm substances to body temperature, if this does not damage the compound, since injection of cold substances can cause discomfort and influences the absorption of the drug.
- If injecting a hazardous substance (biohazard, radiation hazard or chemical hazard), include precautions in the Animal Care Protocol for human safety (e.g. anesthesia, use of restrainer).

COMPLICATIONS:

- **Bleeding at injection site:**
    - ***Cause:*** Nicking of a small skin capillary.
    - ***Clinical Signs:*** A small amount of blood may be seen when the needle is removed from the skin. The bleeding is usually self-limiting.

- ***Response:*** Apply gentle pressure with a cotton tipped applicator or gauze until the bleeding has stopped.

- **Bruising at injection site:**
  - ***Cause:*** Damage to capillaries under the skin (bruising) by the needle at the insertion site or if the skin was held too tightly (pinched). Bruising is usually self-limiting.
  - ***Clinical signs:*** Red to purple skin discolouration at the injection site. Extensive bruising can lead to necrosis or sloughing of the tissue.
  - ***Response:*** If bruising is seen, monitor the tissue daily until it has healed. If any redness or black appearance of tissue is seen, contact your facility veterinarian to determine what treatment is needed.
- **Swelling at injection site:**
  - ***Cause:*** A local reaction to the injected substance. In some cases this is expected but may require intervention.
  - ***Clinical signs:*** Tissue swelling and redness at the injection site.
  - ***Response:*** Monitor daily for worsening signs or that which persists more than 2 days. If so, contact your facility veterinarian. Be prepared to provide analgesia.
- **Lesion formation at injection site:**
  - ***Cause:*** Due to irritation by the injected substance, a larger than recommended volume being injected, or if the animal begins to chew at the area.
  - ***Clinical signs:***
    - Chewing, scratching at site (signs of pain)
    - Redness
    - Infection (moist, purulent discharge)
    - Sloughing or necrosis (blackness) of the tissues surrounding the injection site leading to an open wound
  - ***Response:*** Monitor at least once a day. Refer to Facility Immediate Care and First Aid for Rodents document for treatment. Applying topical antibiotics as soon as redness is seen can help reduce the formation of a lesion or infection. Hibitane or Polysporin creams are good first choices. Contact your facility veterinarian for further treatment options which may include shaving the overlying fur, trimming the hind toenail tips, analgesics, systemic antibiotics and/or other topical treatments of any wounds. Severe wounds or necrosis will require euthanasia.
- **Reaction to injected substance causing clinical signs of illness:**
  - ***Cause:*** A systemic reaction to the injected substance (i.e.: pH is too high or too low, hyper or hypoosmotic, non-sterile).
  - ***Clinical signs:***
    - Loss of weight or body condition
    - Hunching, piloerection, tip-toe gait
    - Decreased activity level
    - Social isolation from cage mates
    - Facial or postural signs of pain
      - Twitching of skin over back (repeatedly)
      - Flinching
      - Staggering when walking (“wobbly”)
      - Freezing behavior followed by sudden movement
      - Licking or scratching at site of injection
      - Squinted eyes

    - Ears curled further back along head (so that tips of ears are farther apart than normal)
    - Whiskers held straight or clumped (loss of normal "curve" of whiskers)
    - Nose and cheek flattening
  - ***Response:*** Increase frequency of monitoring to at least twice a day and provide supportive care such as SQ fluids, easily accessible food and water sources (cage floor level), supplemental heat support, analgesics (if painful), etc. Contact your facility veterinarian for additional treatments. If animal reaches humane endpoint, it must be euthanized.

REFERENCES:

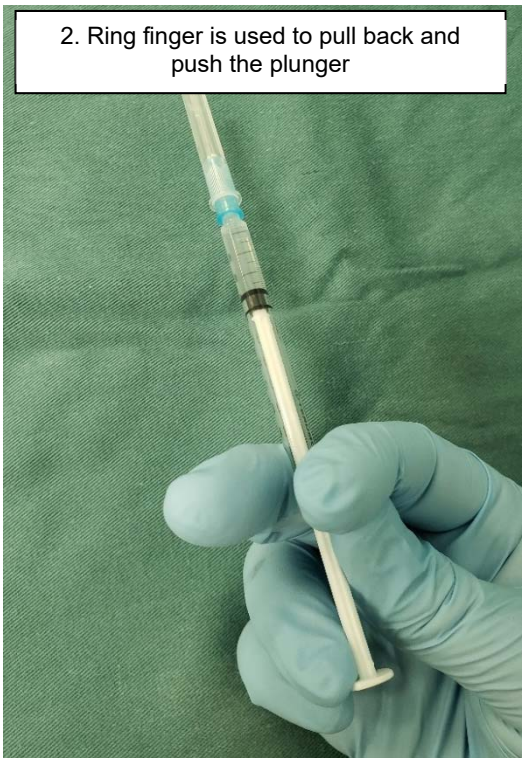
- A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes; Diehl, K et al. 2001 http://onlinelibrary.wiley.com/doi/10.1002/jat.727/abstract
- Administration of Substances to Laboratory Animals: Routes of Administration and Factors to Consider; Turner, Pekow, Vasbinder, Brabb, 2011 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3189662/
- Canadian Council on Animal Care (CCAC) guidelines: mice https://www.ccac.ca/Documents/Standards/Guidelines/CCAC_Guidelines_Mice.pdf
- UBC ACC Guidelines and SOP for the Maintenance of Fluid Homeostasis in Animals

Appendix 1: Examples of ways to hold syringe, for one handed injection

The following figures show 3 ways to keep the syringe/needle steady, when performing injections. Each of these methods makes it possible to pull the plunger back, then push/depress it during injection, without changing your grip on the syringe.

1.Thumb is used to pull back and push the plunger

2. Ring finger is used to pull back and push the plunger



3. Middle finger is used to pull back and ring finger is used to push the plunger