	Animal Health Unit University of Calgary	R14: Rat Injections
Version No.: 4.0		Effective Date: 7/11/2025
No. of pages: 5	Attachments: Y <input type="checkbox"/> N <input checked="" type="checkbox"/>	Superseded Date: 4/19/2022
Level of Invasiveness: B		Prepared by: Animal Health Unit
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R14: Rat Injections

Note: This SOP includes multiple injection methods. When referencing this SOP in your approved Animal Use Protocol (AUP), please ensure you specify precisely which method you are using. If you are varying this SOP in any fashion, you must detail those variations within your approved AUP.

PURPOSE: To outline the acceptable methods of injections for rats.

RESPONSIBILITY: All students, staff and researchers trained by qualified staff under veterinary supervision

PROCEDURE:

General considerations:

- All injections require the handler to perform smooth, decisive movements and skilled animal restraint. Please contact iautp@ucalgary.ca if you require further training on injections and/or animal handling.
- Collect all necessary materials and prepare all syringes before removing the animal from its cage
- All substances to be injected should be near neutral pH, pharmaceutical grade, sterile, pyrogen-free, and warmed to body temperature whenever possible. For further information, refer to SOP ADMp15 Guidelines for the Preparation of Injectable Substances and Agents Administered to Animals.
- Ensure that there are no air bubbles within the solutions in the syringe prior to injection
- Used needles are to be disposed of in sharps disposal containers. Needles **should not** be recapped.
- All injections must be recorded on the animal's cage card and in the Mosaic record keeping system
 - o Include the date, substance injected, volume, dosage, route of injection, and your name
- Animals should be monitored during any injection and after.
- Refinements include using the smallest-diameter needles possible, least painful route, and rotation of injection sites for multiple injections
- While preparing and administering injections, safety goggles are to be worn.

- Recommended volumes and maximum needle gauge (G) sizes in adult (250g) rats are as follows:

	Intraperitoneal	Subcutaneous
Recommended Volume	<10mL/kg	<5mL/kg*
Volume for 250g rat	<2.5mL	<1.3mL
Maximum Needle Size	25 G	25 G

*maximum 2 sites

Prior to any injections, first prepare the equipment:

- Weigh the rat.
- Carefully calculate the appropriate dosage and double check the calculation
- Attach the needle to the syringe.
- Draw up the required volume of liquid for administration based on rat weight, material concentration, and desired dosage.
- With the cap of the needle on, remove any air bubbles by tapping the syringe and repeatedly withdrawing and depressing the plunger.

During and after any procedure, assess the rat for:

- laboured breathing (panting, gasping or open mouth)
- pale or blue instead of pink extremities
- sudden change of behaviour such as hunched posture or reluctance to move
- persistent bleeding at injection site

If any of these occur, stop what you are doing and return the animal to its cage. If the rat's condition does not resolve within a few minutes, contact veterinary staff.

1. Intra-peritoneal Injections (IP)

MATERIALS:

- 30G needle
- Syringe
- Liquid material to administer (of known content and concentration)
- Cage change station or biosafety cabinet

1.1. Restrain the rat as per SOP R8: Rat Manual Restraint.

- 1.1.1. If working alone, restrain using the towel (i.e. burrito (see Figure 1) or alternative towel restraint (see Figure 2) method. Once secure in the towel, expose the landmarks as outlined in 1.1.3. by keeping it tucked close to your body, and roll any excess towel away to expose the landmarks (see Figure 1 and 2)
- 1.1.2. If someone else is available, the partner method of restraint may be used (see Figure 3).



Figure 1: Rat secured in "burrito" hold, flipped with landmarks exposed.



Figure 2: Alternative towel restraint for intraperitoneal injection

- 1.1.3. Identify the correct landmark for injection. The appropriate area for injection is about 1-2mm lateral of the midline, at the intersection of the midline and the hip (see Figure 3).
- 1.2. Tilt the rat at approximately 45° so that its head is at a slight downward angle.
- 1.3. Hold the syringe and needle at a 45° angle from the ventral abdominal skin with the bevel facing upward.
- 1.4. In a single, smooth motion, insert the needle approximately 3-4mm into the abdomen, taking care not to bury the entire needle into the animal (See Figure 4).
- 1.5. Aspirate (gently withdraw the plunger to place negative pressure on the syringe).
 - 1.5.1. If any material (e.g. blood, urine or feces) are seen in the hub of the needle, discard all equipment, monitor the animal for adverse signs and start again if animal appears normal.
- 1.6. If no blood, urine, or feces are seen, proceed with the injection by slowly depressing the plunger to administer the desired volume.
- 1.7. Pull the needle straight back out (on the same angle as for insertion).
- 1.8. Place the syringe and needle into a sharps disposal container.
- 1.9. Monitor the animal for any adverse reactions (see above).



Figure 3: Landmarks for IP injections highlighted in yellow.



Figure 4: Needle inserted into correct location for IP injection. Left: IP injection using a "burrito" method. Right: Restraint using an alternative towel method.

2. Subcutaneous Injections (SQ)

Note: Volumes larger than 5mL should be administered over two separate injections at different body sites.

MATERIALS:

- 30G needle
- Syringe
- Liquid material to administer (of known content and concentration)
- Cage change station or biosafety cabinet
- Saline or hydrogen peroxide
- Gauze

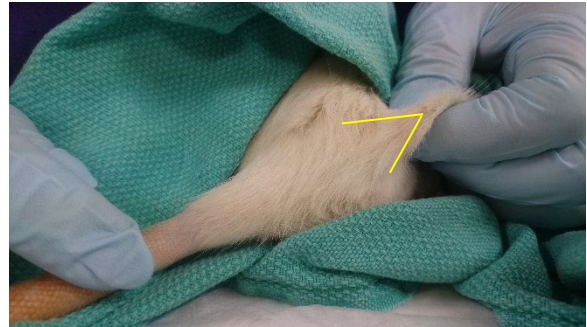


Figure 5: Rat restrained in 'bench-top hold' showing tent of skin over hind quarters, highlighted in yellow

- 2.1. Restrain the rat as per SOP R8: Rat Manual Restraint using the 'Bench-top Hold'.
- 2.2. With the hind quarters exposed, tent a section of skin over the flank or hip area (see Figure 5).
- 2.3. Hold the syringe and needle parallel to the body wall with the bevel facing upward.
- 2.4. In a single, smooth motion, introduce the needle gently into the subcutaneous space at the bottom of the tent (see Figure 6).
- 2.5. Aspirate (i.e. gently withdraw the plunger to place negative pressure on the syringe).
 - 2.5.1. If any material (e.g. blood, urine, feces) are seen in the hub of the needle, pull the needle straight out, discard all equipment, monitor the animal for adverse signs and start again providing the animal is normal.
- 2.6. If nothing is seen on aspiration, proceed with the injection by slowly depressing the plunger to administer the desired volume.
- 2.7. Pull the needle straight back out (on the same angle as for insertion).
- 2.8. Surface bleeding after a subcutaneous injection is common. Apply direct pressure for 30 seconds or until bleeding stops. Contact veterinary staff if the bleeding persists
- 2.9. Clean the injection site of any blood with saline or hydrogen peroxide before returning to the animal to its cage.
- 2.10. Monitor the animal for any adverse reactions (see above).



Figure 6: Correct needle insertion at the bottom of the tent of skin.

REFERENCES:

1. Turner, P. V., Brabb, T., Pekow, C., & Vasbinder, M. A. (2011). Administration of substances to laboratory animals: Routes of administration and factors to consider. *Journal of the American Association for Laboratory Animal Science*, 50(5), 600–613.
2. Turner, P. V., Brabb, T., Pekow, C., & Vasbinder, M. A. (2011). Administration of substances to laboratory animals: Equipment considerations, vehicle selection, and solute preparation. *Journal of the American Association for Laboratory Animal Science*, 50(5), 614–627
3. Morton, D. B., Jennings, M., Buckwell, A., Ewbank, R., Godfrey, C., Holgate, B., Inglis, I., James, R., Page, C., Sharman, I., Verschoyle, R., Westall, L., & Wilson, A. B. (2001). Refining procedures for the administration of substances. *Laboratory Animals*, 35(1), 1–41. <https://doi.org/10.1258/0023677011911345>
4. Research Animal Training. (n.d.). Procedures with care. *Research Animal Training*. Retrieved from <https://researchanimaltraining.com/article-categories/procedures-with-care/>
5. Canadian Council on Animal Care. (2025). *CCAC guidelines on: Rats*. Retrieved from: https://ccac.ca/Documents/Standards/Guidelines/CCAC_Guidelines_Rats-Sept2022.pdf
6. Canadian Council on Animal Care. (2025). *CCAC guidelines on: Scientific procedures Part A*. Retrieved from: https://ccac.ca/Documents/Standards/Guidelines/CCAC_Guidelines-Scientific_procedures-Part_A.pdf