

# UBC ANIMAL CARE COMMITTEE

## TECH 10b - Intraperitoneal Injection in the Adult Rat SOP

Last date revised: November 2020

Date approved: November 2020

Version No. 2

### PURPOSE:

- To describe the procedure for administering intraperitoneal (IP) 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 the intraperitoneal injections.
- All animal users performing intraperitoneal injections in rodents must have successfully completed the UBC Animal Care Services (or equivalent) Introduction to Working with Rodents in Research (IWRR) and Rodent Restraint/SQ/IP injections (RSCIP) courses.

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

- Sterile syringes (1-5 ml)
- Sterile needles (23-25G; 5/8" length or less)
- 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



**Table 1 - RECOMMENDED NEEDLE SIZE AND MAXIMUM VOLUME OF ADMINISTRATION**

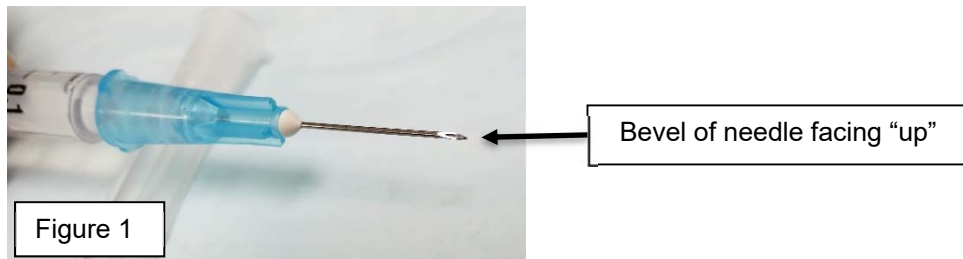
Species	Needle Gauge and Length	Maximum Volume per IP Injection*
Rat	23-25g; 5/8" or smaller	10 ml/kg

\*The lowest volume possible should be administered. 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.

See below for example of how to calculate drug volume.

## 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. Do not overheat beyond 37°C
2. Weigh the animal and calculate the volume to be administered (refer to Table 1 above for maximum recommended volume and below for how to calculate volume).
3. Disinfect the top of the container with alcohol-moistened gauze.
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).

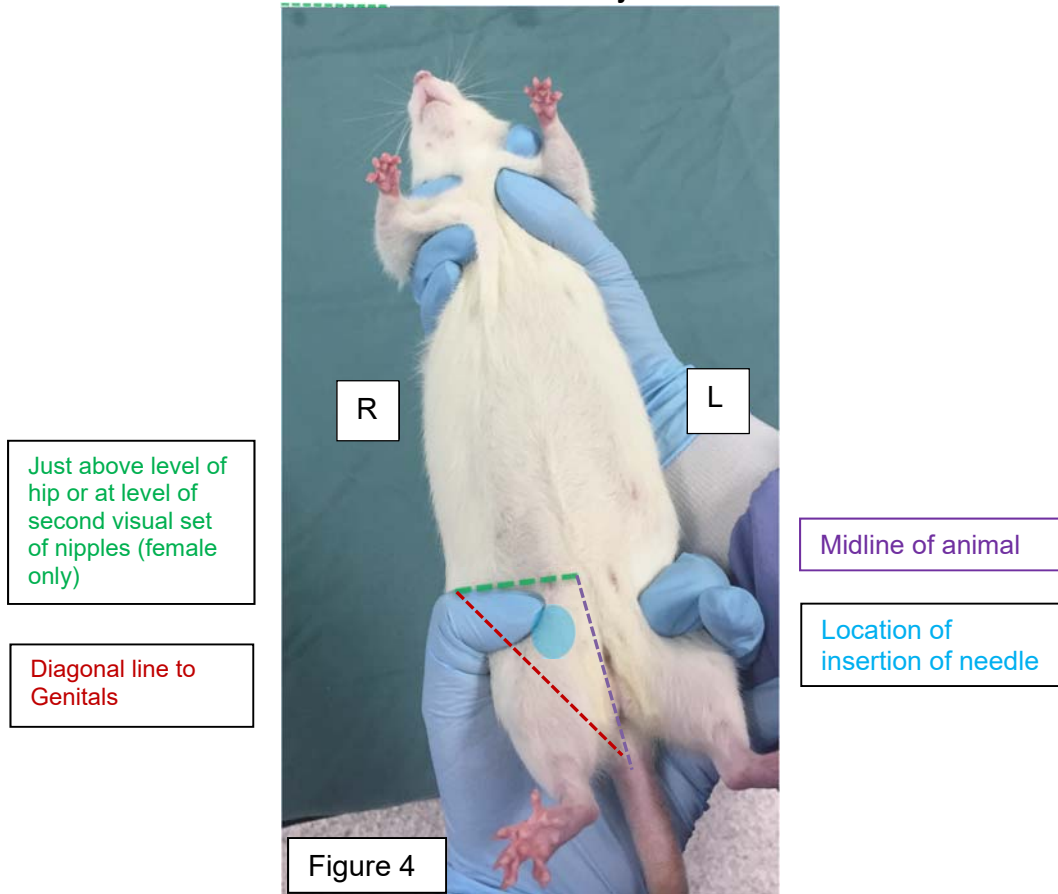


6. The needle can be slid back into the cap loosely without handling either the cap or the needle (see Figures 2 and 3) while you restrain the animal.

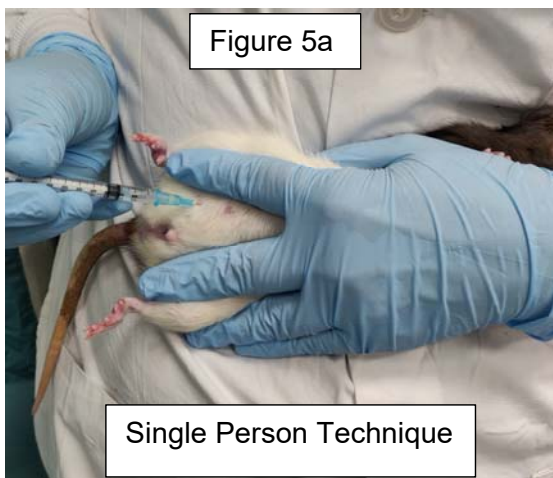


7. Restrain the rat in an appropriate manner.
  - a. See Appendix 1 for single and two-person options for restraint.
  - b. If restraining with a towel, ensure that the weave of the towel allows the rat to breathe once wrapped and that it is not so loose a weave that the rat can catch and tear its toenails.
8. Identify anatomical landmarks to locate the appropriate IP injection site.
  - a. Typically, the injection site is the animal’s **lower right quadrant** of the abdomen to reduce the chances of damage to the urinary bladder, cecum and other abdominal organs (see Figure 4).
  - b. Inject into the centre (blue circle) of the imaginary triangle described in Figure 4.
  - c. If injecting daily for multiple days, it is acceptable to alternate between the right and left sides, if approved on the animal care protocol.

### Landmarks for IP Injection



9. Hold the syringe in your dominant hand to inject.
10. Insert needle into the lower right quadrant of the abdomen with your dominant hand.
  - a. Insert needle at a 30-40° angle to the horizontal plane of the animal with the bevel facing up (see Figures 5a and 5b).
  - b. Direct the needle towards the head of the animal.
  - c. With a 5/8" needle length, approximately  $\sim \frac{3}{4}$  the needle length is inserted. In fat animals, almost the entire length of the needle length may need to be inserted.



11. Pull back on the plunger to ensure negative pressure and that nothing is aspirated (no urine, intestinal contents or blood enters the syringe) prior to injecting. Do not allow the needle to move further in or out of the abdomen while pulling back on the plunger or injecting.
  - a. If aspirate is:
    - Green/brown material, likely the bowel has been punctured.
    - Yellow liquid material, likely the bladder has been punctured.
    - Blood, indicates an abdominal blood vessel has been punctured.
  - b. If contents are aspirated:
    - Withdraw the needle and monitor animal for 5-10 minutes for any signs of clinical illness prior to reattempting injection.
    - The syringe/syringe contents and needle must be discarded and replaced.
    - Record this complication in the monitoring records for this animal.
  - c. If animal appears fine, one more attempt at injecting is permitted. When reinjecting, redirect the angle of the needle slightly and monitor the animal closely for 5-10 minutes for any signs of pain, illness, etc. (see Potential Complications below).
12. If no fluid aspirated, proceed with the injection - depress the plunger until the solution has been fully administered. Remove needle from the abdomen at the same angle as it was inserted.
  - a. The speed of injection depends on the volume and viscosity of the substance. If injecting less than the maximum recommended volumes of aqueous solutions, the injection can be completed in 1-2 seconds. More viscous solutions may take an extra few seconds.
  - b. See suggestions at the end of this document (Appendix 2) on holding the syringe to pull back and inject, without readjusting your hold on the syringe.

**Note:**

The needle must stay in position, once inside the abdomen. If the animal struggles or the person injecting is not able to maintain control of the syringe or animal, remove the needle immediately. If sterility is maintained, the needle can be used for one more attempt. Re-restrain the animal and make a second attempt at the injection. If rat continues to struggle, a different restraint approach may be needed.

13. Discard syringe and needle directly into a sharps container.
  - a. DO NOT RECAP once it has been in an animal.
14. Return the animal to its cage and observe for any complications (see below).
15. Note procedure (drug, dose, route, volume) 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  $\div$  1000 = 0.250kg
- 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 0.250 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 and not exceed the current recommended volume guidelines (see Table 1 above).
- Follow the recommended needle size (see Table 1 above). Using 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 (peritonitis) and/or irritation at the site of injection.
- All substances for injection must be biocompatible since foreign body reactions may lead to peritonitis.
- 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 performing repeated IP injections, (i.e. daily/weekly), start on the right lower quadrant, next move to the lower left quadrant, closer to base of "triangle" (to avoid the cecum). Note where each injection is made and subsequent injections should be made in a slightly different spot than the previous injection.
- If injecting a hazardous substance (biohazard, radiation hazard or chemical hazard), include precautions in the Animal Care Protocol for human safety (e.g. anesthesia).

## 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 to site of bleeding with a clean, dry gauze for 20 seconds. Once bleeding has stopped, gently clean blood off the fur with a wet gauze. Monitor for any signs of swelling at the injection site or abdominal pain (see signs of abdominal pain below). If seen or bleeding continues, contact facility Veterinarian.
- **Signs of abdominal pain:**
  - **Cause:** pH of the drug is not close to neutral, the drug is irritating to the peritoneal lining/abdominal organs, or if the drug is not close to body temperature (too hot or too cold). Signs may be seen either immediately after injection or later if peritonitis develops.
  - **Clinical signs:**
    - Abdominal tucking (sides of abdomen are "sucked in" so that the animal appears to have a waist)
    - Rear leg stretching or lifting

- Abdominal pressing (abdomen is pushed against the cage bottom or side)
  - Hunching (like a cat arching its back) 
  - Piloerection
  - Twitching of skin over back (repeatedly)
  - Flinching
  - Abdominal writhing
  - Staggering when walking (“wobbly”)
  - Freezing behavior followed by sudden movement
  - Licking at abdomen or site of injection
  - Facial Grimace signs
    - 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:** If immediate abdominal pain signs are seen, stop all injections of that drug, provide affected animals analgesia and contact the facility Veterinarian. If abdominal pain signs develop later, provide the animal with analgesia and contact the facility Veterinarian.
- **Peritonitis:**
    - **Definition:** Inflammation or infection of the peritoneal cavity.
    - **Cause:** Intestinal tract damage during an injection, the drug is not biocompatible or is irritating, or if non-sterile substances are injected.
    - **Clinical signs:** Typically show some or all of the abdominal pain signs above as well as systemic clinical signs of illness including:
      - Abdominal pain signs (see above)
      - Weight loss, or abnormal weight gain (with loss of body condition) from fluid formation
      - Lethargy or change in behavior/activity level
      - Piloerection
      - Distension of the abdomen (usually due to fluid (ascites) accumulation in the abdomen)
      - Porphyrin staining around eyes, nose, front paws
      - Signs of dehydration (squinted/sunken eyes, skin tent > 2 seconds, pale extremities)
    - **Response:** If animal is showing any of the above signs, contact the facility Veterinarian.
- **Laceration of abdominal organs, intestine and/or damage to internal blood vessel:**
    - **Cause:** Laceration with the needle at time of injection.
    - **Clinical signs:** Damage to the internal organs or an internal blood vessel will result in bleeding into the peritoneal cavity. If the intestine is lacerated, signs of peritonitis described above will become apparent in 12-48 hours. Occasionally, no blood is seen when the plunger is pulled back prior to injection but a blood

vessel may still be damaged which can result in significant blood loss, hypovolemic shock and death. Signs of internal blood loss include:

- Pale, cold skin and extremities
  - Pale, dry mucous membranes
  - Increased respiratory rate and effort
  - Lethargy
  - Piloerection
- **Response:** If the animal is showing signs of hypovolemic shock (from blood loss), contact the facility veterinarian. SC fluids (LRS) should be given at 20ml/kg, animal placed on a safe heat source and monitored closely until it is acting normally, has normal respiratory effort/rate and its mucous membranes are pink.
- **Injection into the intestinal tract or bladder:**
    - **Clinical signs:** If the injection occurs into the intestinal tract and only a small needle poke is made into the intestine, signs of peritonitis (described above) are unlikely but the drug injected will not be absorbed as expected. If the urinary bladder is punctured (small needle poke), the drug will be injected into the urinary bladder and be urinated out (will not be properly absorbed). If the urinary bladder is lacerated, urine can enter the peritoneal cavity and cause irritation (signs of peritonitis may become apparent). Signs of injection into intestinal tract or bladder include:
      - Peritonitis (clinical signs described above)
      - Failure of drug to be absorbed
      - Blood in the urine (if bladder damaged)
    - **Response:** If animal has signs of peritonitis, contact the facility Veterinarian. If animal has blood in its urine after an IP injection, monitor for additional clinical signs as per “Laceration of abdominal organs” above and continued bloody urine. If these are seen, contact the facility Veterinarian.

## 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: rats  
[https://www.ccac.ca/Documents/Standards/Guidelines/CCAC\\_Guidelines\\_Rats.pdf](https://www.ccac.ca/Documents/Standards/Guidelines/CCAC_Guidelines_Rats.pdf)
- UBC ACC Guidelines and SOP for the Maintenance of Fluid Homeostasis in Animals for volumes.

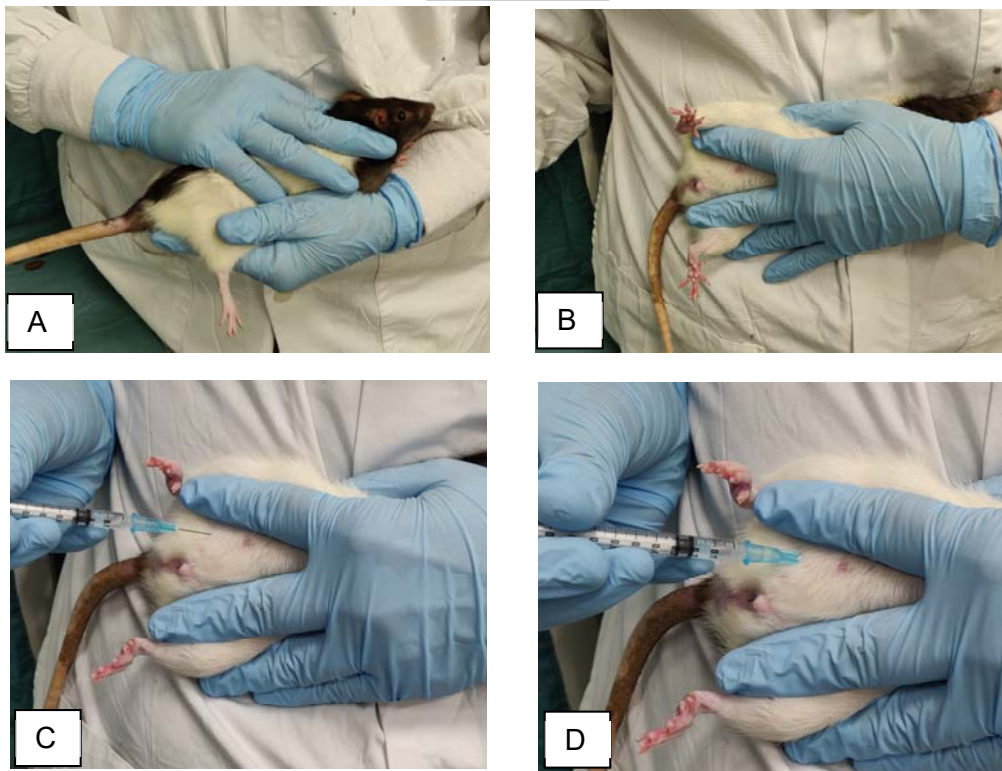


## Appendix 1: Restraint and position of animal for IP injection.

### Option 1 - Single Person Restraint, against body technique: Preferred method. See Figure 6.

- Most rats stay calmer and relax more quickly with this type of restraint, rather than the typical 2-Person V-hold method.
- Support the rat on your non-dominant arm, facing your elbow, with the hand along its abdomen (Figure 6A).
- With your dominant hand/wrist, press along the rat's back to keep it supported, roll the rat so that its dorsal surface (back) is against your abdomen and press rat against your body as you remove your dominant hand.
- Keep the rat in place against your abdomen, by keeping your non-dominant hand straight and applying enough pressure to keep it in place and securely supported.
- Adjust your fingers so that you are able to control the upper hind leg while ensuring it cannot move during the IP injection, and using the middle or ring finger, hold the lower hind leg out of the way (Figure 6B).
- Ensure the rat's body is straight and tilted in a slightly head down position just prior to injecting.
- Identify the proper landmark for injection (Figure 6C)
- Insert the needle at a 30-40° angle to the horizontal plane of the animal with the bevel facing up and perform the injection.

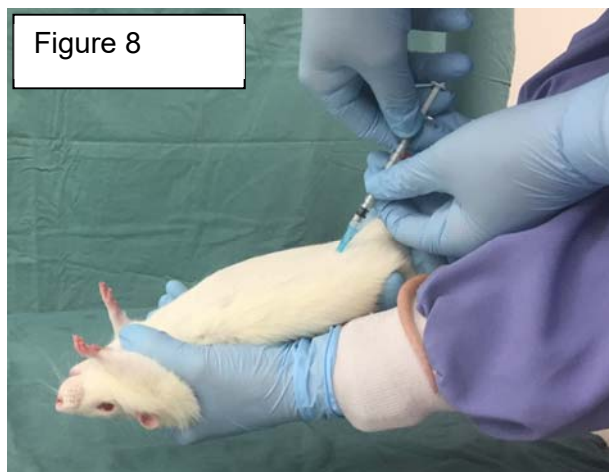
Figure 6





**Option 2 - The 2-person technique:** may use in a BSC or when unable to hold rat against body

- Person 1: Use the scissor or 'V' hold with your dominant hand (See Figures 7 and 8).
  - Hold the rat's head between your index and middle fingers of your dominant hand without pressing on the trachea.
  - Wrap the remaining fingers gently around the thoracic cavity without squeezing the animal.
  - Straighten your index and middle finger to extend the head and further restrain the rat.
  - With your other hand, hold onto the rear legs and tail.
    - Place your hand along its back to support the hind end
    - Position your thumb into the groin on one side and your middle finger into the groin on the other side.
    - Once in position, gently, pull back to stretch/straighten the back legs and open the hips so that there is space for the person to see where they are injecting.
    - Gently tilt the head slightly lower than the body before the second person performs the injection.
- Person 2: Communicate with the person who is restraining the rat. As soon as they indicate they have a secure restraint, be ready to perform the injection.
  - Identify the proper landmark for injection.
  - Insert the needle at a 30-40° angle to the horizontal plane of the animal with the bevel facing up and perform the injection.



## Appendix 2: Examples of ways to hold syringe, for one handed injection

The following figures show ways to keep the syringe/needle steady, when performing injections (to assess for negative pressure or when ensuring safe needle placement in the intraperitoneal space). Each of these methods, makes it possible to pull the plunger back, then push/depress, during injection, without changing grip on the syringe.

