



Standard Operating Procedure: Intravenous Injections in the Rat

These SOPs were developed by the Office of the University Veterinarian and reviewed by Virginia Tech IACUC to provide a reference and guidance to investigators during protocol preparation and IACUC reviewers during protocol review. They can be used as referenced descriptions for procedures on IACUC protocols.

However, it is the sole responsibility of the Principal Investigator to ensure that the referenced SOPs adequately cover and accurately represent procedures to be undertaken in any research project. Any modification to procedure as described in the SOP must be outlined in each IACUC protocol application (e.g. if the Principal Investigator plans to use a needle size that is not referenced in the SOP, simply state that alteration in the IACUC protocol itself).

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I. Procedure Summary and Goal

Describes procedure for the administration of fluids or compounds intravenously.

Considerations:

- a. Provides the fastest absorption rate, as fluids are administered directly into the venous system.
- b. Primary intravenous (IV) injection site for the rat is the right and left lateral tail vein. Other sites (e.g., retro-orbital [RO] orbital sinus) require sedation and/or analgesia).
- c. Please refer to the Guidelines for Injections in Rodents and Rabbits, Virginia Tech Office of the University Veterinarian for recommended volumes and needles sizes.
 - i. Tail Vein: 0.5ml
 - ii. Retro-orbital Sinus: <0.15ml

II. Personal Protective Equipment (PPE) and Hygiene

- a. Ensure appropriate PPE is used to protect technician from accidental exposure to blood and other body fluids, such as:
 - i. Gloves
 - ii. Eye protection
 - iii. Mask
 - iv. Other PPE as required by protocol/facility
- b. Hands should be washed and/or gloves changed between animals.
- c. Promptly dispose of used sharps in the provided leak-proof, puncture resistant sharps container.

III. Supply List

- a. Mechanical restraint device
- b. Needles (21-23 gauge; ½ - ⅝ inch)
- c. Prefilled syringes
- d. Heat source (e.g., heating pad, warm water bath or heat lamp)
- e. Antiseptic solution
- f. Gauze pads
- g. Anesthetic agents
 - i. Inhalant
 1. Isoflurane – inhaled to effect
 2. 70% CO₂ + 30% O₂ Gas – inhaled to effect
 - ii. Injectable

1. Ketamine (90-120mg/kg) + Xylazine (8-12mg/kg) combination
 - a. Intramuscular, subcutaneous, or intraperitoneal injection
- iii. Topical ophthalmic anesthetic (e.g., Proparacaine Hydrochloride Ophthalmic Solution)
- h. Ophthalmic ointment
- i. Clotting agents (e.g., styptic powder, silver nitrate sticks, cautery pen)

IV. Detailed Procedure

- a. Anesthesia
 - i. Tail vein – no anesthesia required
 - ii. Retro-orbital sinus – general anesthesia prior to injection. Additionally, apply a single drop of topical ophthalmic anesthetic (Proparacaine Hydrochloride) prior to procedure. Allow a minimum of 30 seconds for the medication to take effect prior to injection.
- b. Procedure
 - i. Tail Vein IV Injection

The rat has two lateral tail veins and a tail artery located on the ventrum of the tail (Figures 1 and 2).

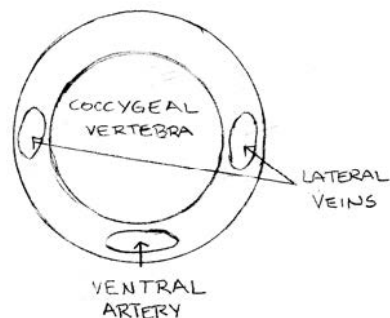


Figure 1. Cross-section Schematic of Tail

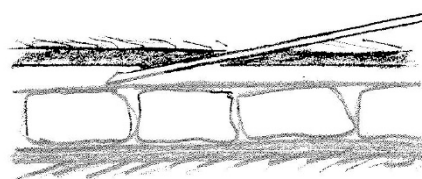


Figure 2. Transection Schematic of Tail Showing Needle Placement

- ii. Restrain the animal using the mechanical restraint device of your choice with the tail protruding (Figure 3).
- iii. Warming the rat (or tail only) causes vasodilation and provides better vein visibility. This may be done using a heating chamber, heating pad or a heating lamp, or dipping tail in warm water.

CAUTION: do not overheat; animal should be under a heat lamp no longer than three minutes with heat lamp at least 12 inches away.



Figure 3. Mechanical Restraint

- iv. Wipe tail with antiseptic solution.

- v. Immobilize the tail with the non-dominant hand and rotate $\frac{1}{4}$ turn to access the lateral tail vein (Figure 4).
- vi. Align the needle parallel to the tail with the beveled edge of the needle facing up.
- vii. Insert needle into vein starting at the tip of the tail (distally) at about a 30° angle. Blood may flash to the hub, or gently aspirate to confirm blood flow (Figure 5).

NOTE: If placement is successful, the vein will blanch and compound will flow easily during administration. In an unsuccessful injection, the material will not move through the vein and there will be resistance. If proper placement cannot be confirmed, attempt to place the needle in a more proximal position (i.e., towards the base of the tail). The vein is deeper the closer you move towards the body and can be more difficult to access.

- viii. Administer compound slowly and evenly, and remove needle when complete.
- ix. Apply gentle pressure with gauze (15-30 seconds) until bleeding has stopped. Clotting agent may be used to facilitate the stop of blood flow.
- x. Dispose of the needle into the approved Sharps container.

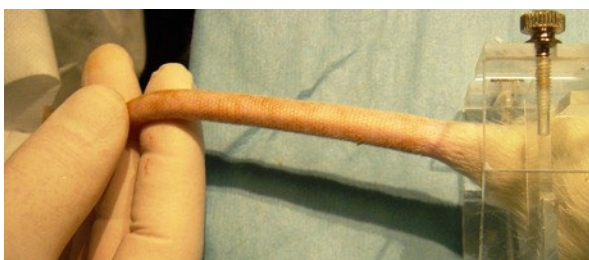


Figure 4. Visualize Lateral Vein

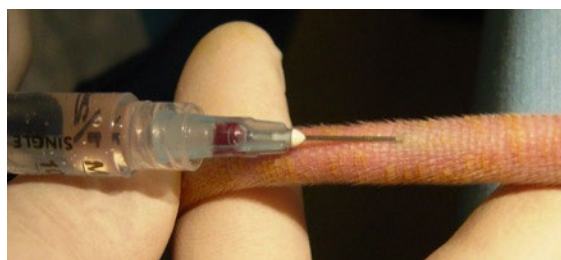


Figure 5. Observe Blood Flash

c. Retro-orbital Plexus IV Injection

- i. Considerations
 1. Utilize small gauge needle and syringes (e.g., tuberculin syringe with 27 g needle).
 2. Volume to be injected should not exceed 0.15ml.
- ii. Anesthetize the animal with the selected anesthetic agent prior to sample collection procedures.
- iii. Apply a single drop of topical ophthalmic anesthetic (Proparacaine Hydrochloride) prior to procedure. Allow a minimum of 30 seconds for the medication to take effect prior to injection (Figure 7).
- iv. Place animal in lateral recumbency, and scruff the animal such that the eyeball partially protrudes from the socket.
- v. Introduce the needle, bevel down so as to not damage the surface of the eye, into the medial canthus of the eye at a 45° angle, between the globe and the eye bony orbit (Figure 8).
- vi. Advance needle following the edge of the eyeball down until the needle tip is at the base of the eye.

- vii. Slowly inject, do not aspirate; once administration complete, slowly withdraw needle.
- viii. Observe animal for full anesthetic recovery.



Figure 6. Apply Topical Anesthetic



Figure 7. Insert Needle into Medial Canthus of Eye

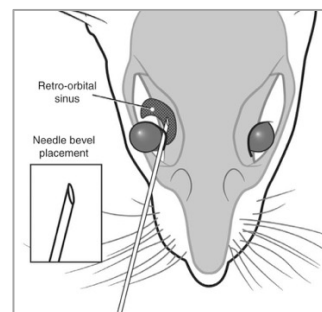


Figure 8. Correct placement of the needle relative to the retro-orbital sinus, the eyeball the back of the orbit. Illustration by Darryl L Lab Anim (NY). 2011 May; 40(5): 155-160

V. Variations

None

VI. Potential Adverse Events, Mitigation, or Treatment

- a. Potential adverse effects related to tail vein injections
 - i. Tail necrosis secondary to extravasation of injected substances
 - ii. Bruising, hematoma, continued bleeding
 - 1. Apply pressure until active bleeding has stopped
 - 2. Recheck within 24 hours
 - iii. Distress due to hyperthermia if animal is warmed
- b. Potential adverse effects related to retro-orbital injections
 - i. Anesthetic respiratory distress
 - ii. Eye infection, loss of eye, blindness
 - iii. Peri-orbital swelling, redness and/or hematoma formation
 - iv. Death

VII. References

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