



Standard Operating Procedure: Blood Collection from the Tail Vein in Rats

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 procedures for the collection of small blood samples from the tail vein (common vascular access route that requires no anesthesia) as a survival procedure in the rat.

Considerations:

- a. The rat has two lateral tail veins and a tail artery located on the ventrum of the tail (Figure 1). A greater volume of blood may be collected from the artery, but care must be taken to ensure adequate hemostasis, and therefore should only be used if larger amounts are needed.
- b. When prepping tail and/or during sample collection, do not “milk” the tail or rub back and forth as this may affect sample quality (e.g., tissue or blood cell damage, contamination).
- c. 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.
- d. Packed cell volume (PCV) and hemoglobin measurements have been reported to be higher in blood collected from the tail vein compared to blood collected from other sites.
- e. Please refer to the Guidelines for Injections in Rodents and Rabbits, Virginia Tech Office of the University Veterinarian for recommended volumes and needles sizes.
- f. Blood volume collection determination (ARAC Guidelines)
 - i. The total circulating blood volume of a rodent is estimated to be approximately 8% of body weight.
 - ii. Of the circulating blood volume, approximate percentages of the total volume which can safely be removed are as follows:
 1. 10% every two to four weeks
 2. 7.5% every seven days
 3. 1% every 24 hours.

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. Appropriate collection tubes (i.e. heparinized or non-heparinized micro-hematocrit tubes, micro-centrifuge tubes)
- c. Capillary tube sealant, for example Crito-O-Seal®
- d. 22 gauge or smaller needles, syringes (e.g., 1cc tuberculin, 3cc)
- e. Heat source (e.g., heating pad, warm water bath or heat lamp)
- f. Antiseptic solution
- g. Gauze
- h. Optional
 - i. Topical anesthetic
 - ii. Scalpel blade (for tail nick or tail snip)
 - iii. Clotting agents (e.g., styptic powder, silver nitrate sticks, cautery pen)

IV. Detailed Procedure

- a. Frequency
 - i. The tail vein can be used for repeated or serial blood collection.
- b. Anesthesia
 - i. This method can be performed without anesthesia when using an effective restraint device.
- c. Procedure
 - i. Restrain the animal using the mechanical restraint device of your choice with the tail protruding.
 1. Visualize anatomic landmarks (Figures 1 and 2)

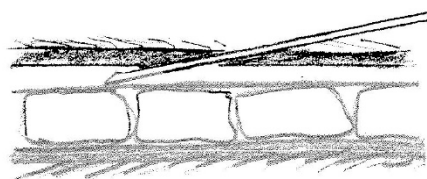


Figure 1. Transection Schematic of Tail

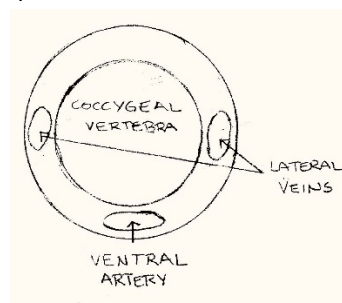


Figure 2. Cross-Section Schematic of Tail

- ii. Cleanse collection site with antiseptic solution; do not rub back and forth to prevent sample quality degradation.
- iii. Immobilize the tail with the non-dominant hand and rotate $\frac{1}{4}$ turn to access the lateral tail vein.
- iv. Align the needle parallel to the tail with the beveled edge of the needle facing up.

- v. Insert needle into vein starting at the tip of the tail (distally)
 1. Gently aspirate to collect via syringe (Figure 3);
 2. Observe blood flash and allow blood to drip from needle hub into collection tube or into hematocrit tube via capillary action (Figure 4);
 3. Remove needle and collect droplets of blood into the collection tube or into hematocrit tube via capillary action.
- vi. Apply gentle pressure with gauze 15 to 30 seconds until bleeding has stopped. Clotting agent may be used to facilitate the stop of blood flow.
- vii. Dispose of the needle into the approved sharps container.
- viii. If anesthetized, monitor animal until fully awake and able to walk normally.
- ix. Repetitive bleeds may be performed by inserting the needle further up the tail.

NOTE: the vein is deeper the closer you move towards the body [proximally] and can be more difficult to access.



Figure 3. Blood Flash into Needle Hub

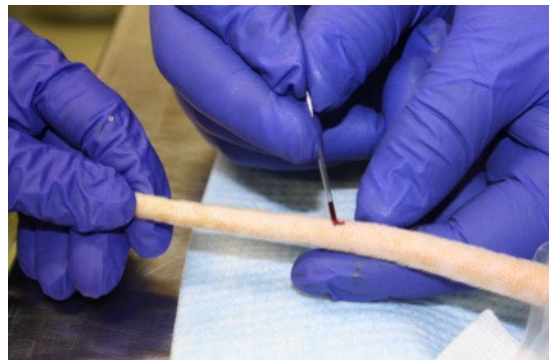


Figure 4. Using a Hematocrit Tube

V. Variations

- a. Tail Nick
 - i. Following step IV.c.3, prick the vein perpendicular to the tail as close to the tail tip as possible.
 - ii. Collect blood via capillary action using hematocrit tube or allowing blood to drip into collection tube.
 - iii. Ensure good hemostasis by applying pressure (30-45 seconds) with a dry gauze pad; clotting agents or surgical glue may be used if needed.
- b. Tail Clip (Amputation)
 - i. Considerations:

1. No more than 2mm of distal tail for rats to be removed. Only the fleshy portion of the tail should be cut, not the vertebrae. If tail previously sampled for genotyping, this technique may not be allowed.
 2. Topical anesthetic required; general anesthetic recommended
 - a. If topical hypothermic anesthetic used, blood flow will increase as tail re-warms
 - b. If local anesthetic used, allow adequate contact time.
 3. Repeated serial collections are possible by gently removing the clot/scab for each sample.
- ii. Following step IV.c.3, using scalpel blade (or sharp scissors), cut 1-2mm of the distal tail at an angle perpendicular to the tail.
 - iii. Ensure good hemostasis by applying pressure (30-45 seconds) with a dry gauze pad; clotting agents or surgical glue may be used if needed.

VI. Potential Adverse Events, Mitigation, or Treatment

- a. Distress due to restraint, overheating, blood loss
 - i. Release restraint, gently stimulate mouse until it recovers and is walking
 - ii. Contact veterinary staff if animal does not recover normally
- b. Hematoma, local trauma, infection, or irritation at blood collection site
 - i. Contact veterinary staff

VII. References

American Association of Laboratory Animal Science. Laboratory Animal Technician Training Manual. (Memphis, TN: Drumwright and Co, 2007)

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