



Standard Operating Procedure: Mouse Blood Collection from the Submandibular Vein (Cheek Punch)

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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Procedure Summary and Goal

Describes procedures for the collection of small blood samples from the cheek (submandibular) as a survival procedure in the mouse.

Considerations:

Variable sample quality; may be a mixture of venous and arterial blood.

Clinical chemistry values may be higher with this method than with retro-orbital collection.

Please refer to the Guidelines for Injections in Rodents and Rabbits, Virginia Tech Office of the University Veterinarian for recommended volumes and needles sizes.

Blood volume collection determination (ARAC Guidelines)

- a. The total circulating blood volume of a rodent is estimated to be approximately 8% of body weight.
- b. Of the circulating blood volume, approximate percentages of the total volume which can safely be removed are as follows:
 - i. 10% every two to four weeks
 - ii. 7.5% every seven days
 - iii. 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.
 - 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. Appropriate collection tubes (e.g., heparinized or non-heparinized microcentrifuge tubes)
- b. Lancet
 - i. 4mm for a small/young mouse
 - ii. 5mm to 5.5mm for an average adult mouse



- c. Gauze pads

IV. Detailed Procedure

- a. Frequency
 - i. The submandibular vein can be used for serial blood collection of small samples by alternating cheek sides, and/or by gently removing the clot/scab.
- b. Anesthesia
 - i. This method can be performed without anesthesia, but does require training to perfect technique.
- c. Procedure
 - i. Properly restrain the animal with one hand, which will apply adequate pressure to the maxillary vein.
 - ii. Visualize landmarks
 - 1. Albino mice have a convenient landmark in the area of the maxillary vein. Often viewed as a small bald spot or hair whorl, you can find this along the curvature of the mandible; the vein is just below this mark in the groove past the jawbone. Darker mice have the same spot but it may be more difficult to see.
 - 2. You can also align a lancet caudal to the angle of the mandible, extending to the ear. Location of the puncture site is at the midway point of the lancet.
 - iii. Using the point of the lancet, apply firm pressure at the point, caudal to the eye and ventral to the ear, where the submandibular vein is located, then release until blood flows.
CAUTION: hold needle or lancet perpendicular to the bleed site to avoid puncturing the ear canal (Figure1).



Figure 1



Figure 2

- iv. Quickly place blood collection tube below the puncture site until desired volume of blood is collected (Figure 2).
- v. Apply gentle pressure with gauze until bleeding has stopped.

- vi. Dispose of the needle/lancet into the approved sharps container

V. Variations

Use a 20-gauge needle to puncture vein; leave in place and allow blood to drip into collection vial.

VI. Potential Adverse Events, Mitigation, or Treatment

- a. Distress due to restraint or blood loss
 - i. Release restraint, gently stimulate mouse until it recovers and is walking
 - ii. Contact veterinary staff
- b. Puncture of ear canal due to improper technique
 - i. Apply pressure with gauze until bleeding stops
 - ii. Contact veterinary staff
- c. Local trauma, hematoma, infection, or irritation at blood collection site
 - i. Contact veterinary staff

VII. References

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