



Standard Operating Procedure: Blood Collection from the Lateral Saphenous Vein in the Mouse

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 lateral saphenous (common vascular access route that requires no anesthesia) as a survival procedure in the mouse.

Considerations:

This can be done on a non-anesthetized animal, but effective restraint is required.

Variable sample quality.

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, 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, 50ml syringe tube with holes drilled for breathing
- b. Appropriate collection tubes (e.g., heparinized or non-heparinized micro-hematocrit tubes, microcentrifuge tubes)
- c. Capillary tube sealant, for example Crito-O-Seal® (optional for procedure)
- d. Needles (22 gauge or smaller) or lancet (4 – 5 mm)
- e. Petroleum jelly or ophthalmic ointment

- f. Gauze pads

IV. Detailed Procedure

- a. Frequency
 - i. The saphenous vein can be used for serial blood collection of small samples by gently removing the clot/scab or puncturing the vein proximal to the initial site.
- b. Anesthesia
 - i. This method can be performed without anesthesia when using a restraint device.
- c. Procedure
 - i. Restrain the animal using the mechanical restraint device of your choice with the head covered and the hind leg accessible.
 - ii. Remove hair from the lateral side of the hind leg or use petroleum jelly to gently separate the hair.
 - iii. Application of petroleum jelly or ophthalmic ointment prevents blood migration into surrounding hair and increases visualization of vein.
 - iv. Apply mild pressure by cupping the mouse's body within the restraint device with the palm of the hand, and manipulate the leg between two fingers to occlude the saphenous vein.
 - v. Grasp the skin and thigh to extend leg.
 - vi. Using a lancet or needle, gently puncture the vein (Figure 1).
 - vii. Collect drops of blood with capillary tube or collection tube.
 - viii. Apply gentle pressure with gauze until bleeding has stopped.
 - ix. Dispose of the needle/lancet into the approved sharps container.

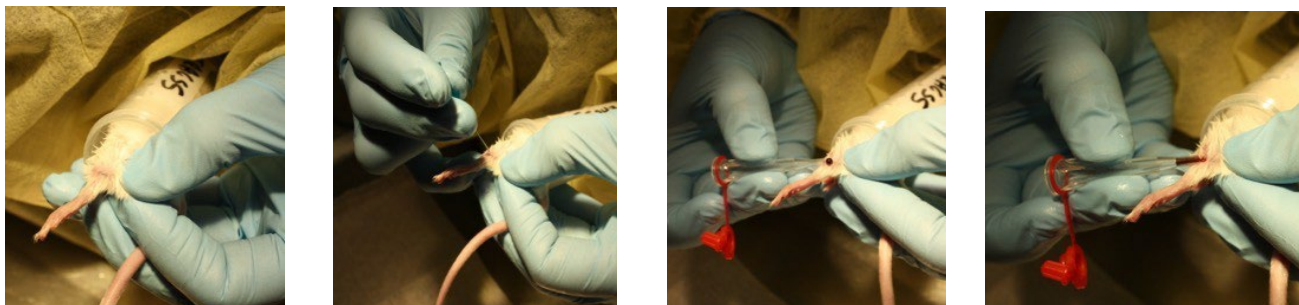


Figure 1. Restraint and blood collection via the lateral saphenous vein

V. Variations

Use of needle to puncture vein as opposed to lancet.

Larger animals may need one person to restrain and another to collect blood.

VI. Potential Adverse Events, Mitigation, or Treatment

- a. Temporary favoring of limb may be noted following procedure
 - i. Recheck animal within 24 hours
 - ii. Contact veterinary staff if this does not resolve
- b. Distress due to restraint
 - i. Release restraint, gently stimulate mouse until it recovers and is walking
 - ii. Contact veterinary staff if animal does not recover normally
- c. 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)

Charles River Insourcing Solutions. Bi methodology of the Laboratory Mouse Charles River SOP 2405-3 - Dosing of Rodents – TGS and Discovery Services Charles River SOP 2577-2 - Blood Collection Methods for Use in Studies

Hawk, C.T., Leary, S.T., and Morris, T.H. Formulary for Laboratory Animals (3rd ed.). (Ames, Iowa: Blackwell Publishing, 2005) http://www.research.usf.edu/cm/docs/Formulary_for_Lab_Animals_3rd_ed.pdf

Hoof, J. Methods of Blood Collection in the Mouse. Lab Animal (NY). 29(10). (2000 Nov)

The Norwegian Reference Centre for Laboratory Animal Science & Alternatives. Saphenous vein puncture for blood sampling of the mouse (nd).<http://film.oslovet.veths.no/saphena/>

Suckow, M., Danneman, P., and Brayton, C. The Laboratory Mouse. (Boca Raton, FL: CRC Press LLC, 2001)