

## **SOP: Blood Collection from the Marginal Ear Vein in the Rabbit**

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 collection of blood via the marginal ear vein in rabbits.

Considerations

- a. One of the most common and least invasive methods of blood collection in the rabbit. The auricular artery can also be accessed for larger amounts of blood, but there is a greater risk of hematoma or bruising.
- b. Easily performed without general anesthesia, but proper restraint and topical anesthetic and/or sedation preferred.
- c. Use of a butterfly catheter for collection reduces negative pressure on the vessel.
- d. Blood volume collection determination
  - i. See Guidelines for Regulating the Volume of Experimental Blood Sample Withdrawals in Laboratory Animals
    - a. Circulating blood volume (CBV) should be determined from known species specific volume to weight values and not calculated based on flat percentage of body weight
    - b. A maximum survival bleed not exceeding 10% of CBV is allowable once monthly
    - c. Bleedings performed weekly should not exceed 7.5% of CBV
    - d. Animals being bled daily may have 1% of CBV taken
    - e. Exceptions to these numbers are possible with fluid replacement therapy
  - ii. *Rabbit Estimated Circulating Blood Volume = 56ml/kg (44-70ml/kg)*
    - a. *Example (3kg rabbit):*  $CBV = 3\text{kg} \times 56\text{ml/kg} = 168\text{ml}$ . Maximum daily withdrawal (without fluid supplementation) =  $0.01 \times 168\text{ml} = 1.7\text{ml}$

**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. Restrainer
- b. Syringes
- c. Blood collection tubes (e.g., vacutainers, hematocrit tubes)
- d. Needles (23-25 gauge;  $\frac{5}{8}$  - 1 inch) or butterfly catheters, or vacutainer needles with hub
- e. Clippers with clean sharp blades
- f. Topical anesthetic
- g. Topical vasodilator
- h. Sedation (e.g. acepromazine) optional
- i. Gauze pads

**IV. Detailed Procedure**

- a. Frequency
  - i. Serial samples can be collected by moving towards the base of the ear on the same vein, or alternating ears. This is also dependent upon the volume of blood collected at each sampling.
- b. Anesthesia
  - i. No anesthesia needed, but adequate restraint required
  - ii. Topical anesthetic cream may be applied to surface of ear for approximately 10 minutes prior to venipuncture
  - iii. Acepromazine administered at 1mg/kg body weight (intramuscular) 20 minutes prior to venipuncture provides sedation as well as vasodilation.
- c. Procedure
  - i. Restrain rabbit securely with mechanical rabbit restrainer ([Figure 1](#)), towel wrap method, or commercially available sacks.

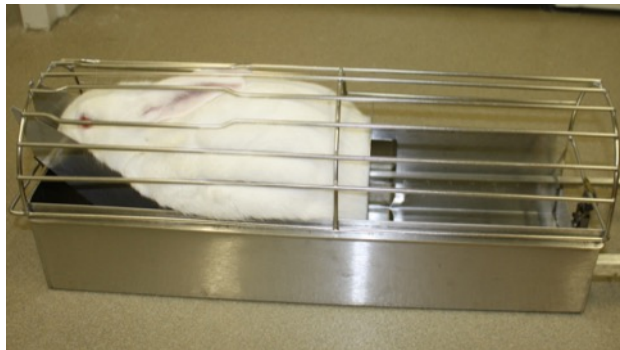


Figure 1. Mechanical Restrainer

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- ii. Shave or pluck hair over the marginal ear vein (Figure 2)

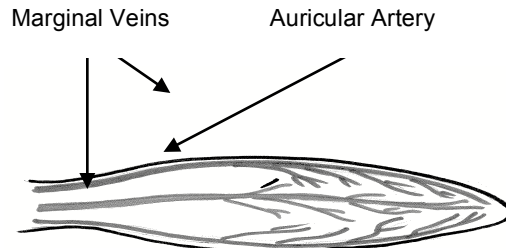


Figure 2. Schematic of Auricular Vessels

- iii. Clean site with antiseptic. Apply local anesthetic cream and allow skin contact for a minimum of 10 minutes.
- iv. To dilate vessel:
  - a. Apply warm compress to ear, or
  - b. Massage ear for 30 to 60 seconds.
- v. Occlude vessel proximal to collection site.
- vi. Holding the ear flap in the non-dominant hand, insert needle, bevel up, into the vein.
- vii. Watch for flash of blood in hub, and maintaining control of the needle, slowly aspirate syringe to prevent vessel collapse (Figure 3).
- viii. Remove needle, and apply firm pressure with gauze for one minute to ensure hemostasis.
- ix. Dispose of the needle into approved sharps container.



Figure 3. Blood Collection

### V. **Variations**

Use a butterfly catheter with syringe or with vacutainer collection tubes.

Nick vein with needle and collect blood through capillary action with hematocrit tube

### VI. **Potential Adverse Effects, Mitigation, or Treatment**

- a. Hematoma or thrombus
  - i. Enter vessel at an angle of 30 degrees or less
  - ii. Use a gauge of needle smaller than the vein
  - iii. Apply pressure until bleeding has stopped (1+ minutes)
- b. Pain at blood collection site

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- i. Use a needle of smaller gauge than the vein
- ii. Practice on vein models prior to live animal
- c. Infection at blood collection site
  - i. Use sterile single-use devices only
  - ii. Clean work surfaces with disinfectant
  - iii. Wear gloves, wash hands
  - iv. Contact a qualified veterinarian for treatment recommendations if any of the following are noted.
    - a. Heat, pain, swelling first noted at the insertion site of the blood draw, purulent material draining from the insertion site.
    - b. Induration (hardening) of the vessel
    - c. Pyrexia, local or systemic infections, septic shock
- d. Syncope
  - i. Contact veterinary staff immediately

## VII. **References**

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