



Standard Operating Procedure: Blood Collection in the Horse

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).

Table of Contents

- I. Procedure Summary and Goal2
- II. Personal Protective Equipment (PPE) and Hygiene2
- III. Supply List3
- IV. Detailed Procedure3
- V. Variations4
- VI. Potential Adverse Effects, Mitigation, or Treatment.....4
- VII. References4

I. Procedure Summary and Goal

Describes procedures for blood collection via the jugular vein in horses.

Considerations:

Refer to SOP: Horse Restraint for methods of restraint

Horses are generally tolerant of blood collection procedure. If a horse is head shy or difficult to restrain, a twitch may provide adequate control. Handlers should be vigilant at all times so as to avoid injury to animals or themselves.

- a. Blood volume collection determination
 - i. See Guidelines for Regulating the Volume of Experimental Blood Sample Withdrawals in Laboratory Animals
 1. 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
 2. A maximum survival bleed not exceeding 10% of CBV is allowable once monthly
 3. Bleedings performed weekly should not exceed 7.5% of CBV
 4. Animals being bled daily may have 1% of CBV taken
 5. Exceptions to these numbers are possible with fluid replacement therapy
- b. Horse Estimated Circulating Blood Volume = 75ml/kg
 - i. Example: 500kg horse: $CBV = 500\text{kg} \times 60\text{ml/kg} = 37,500\text{ml}$. Maximum daily withdrawal (without fluid supplementation) = $0.01 \times 37,500 = 375\text{ml}$

II. Personal Protective Equipment (PPE) and Hygiene

- a. Ensure appropriate PPE is used to protect handler from accidental injury or exposure to blood and other body fluids, such as:
 - i. Scrubs or coveralls
 - ii. Steel-toed shoes or boots
 - iii. Optional
 - iv. Disposable gloves (e.g., latex, nitrile)
 - v. Eye protection
 - vi. Other PPE as required by protocol/facility
- b. Hands should be washed and/or gloves changed between animals; if vacutainer hubs used, ideally, they should also be disinfected between barns or animal holding areas.
- c. Promptly dispose of used sharps in the provided leak-proof, puncture resistant sharps container.

III. Supply List

- a. Needles (18 gauge X 1 ½ inch) or vacutainer needles and hub
- b. Syringes
- c. Blood collection vials
- d. Restraint (e.g., halter, twitch)
- e. Clippers (optional)
- f. Antiseptic
- g. Gauze

IV. Detailed Procedure

- a. Restrain animal; typically, one handler manages the horse while a second handler performs blood collection.
- b. Clip (optional) and swipe with antiseptic gauze to remove superficial dirt and debris. This may also assist in visualizing raised vein.
- c. Occlude jugular vein by applying pressure in the jugular groove 2-3 inches below venipuncture site and visualize raised vein.
- d. With bevel up, insert needle firmly and smoothly (don't jab) through the skin and into the vein at 20° angle.

- i. When using needle and syringe, break the seal on the syringe by gently pulling back before use. Clear air, and with the needle attached to syringe, insert the needle, and aspirate the syringe to confirm insertion and to collect blood (Figure 1).
- ii. If using vacutainer, once needle inserted, stabilize needle and push the vacutainer tube into hub. If you have hit the vein, blood will flow freely into tube. Multiple tubes can be filled by removing filled tube and replacing with fresh tube.

NOTE: Do not pull needle out of vein with vacutainer tube still attached as this will release the vacuum in the vacutainer.



Figure 1. Occlude Jugular Vein by Applying Pressure at Base of Jugular Groove and Insert Needle; Aspirate to Collect Blood

- e. If you have missed the vein, you can carefully reposition needle until vessel penetrated. The vessel may be deep and may roll away from needle. Typically no more than two to three attempts should be made at a time to minimize distress to the animal and potential damage to the vein.
- f. Remove needle and dispose of in approved sharps container.
- g. In order to ensure adequate hemostasis, apply pressure with gauze for one (1) minute.

- h. Serial samples can be taken by alternating sides, and by moving insertion sites cranially, as long as there is no hematoma formation.

V. Variations

- a. Transverse facial vein
- b. Cephalic Vein
- c. Saphenous vein

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
 - 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.
 - 1. Heat, pain, swelling first noted at the insertion site of the blood draw, purulent material draining from the insertion site.
 - 2. Induration (hardening) of the vessel
 - 3. Pyrexia, local or systemic infections, septic shock

VII. References

American Association of Laboratory Animal Science. Assistant Laboratory Animal Technician Training Manual. (Memphis, TN: Drumwright and Co, 2012)

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