



Standard Operating Procedure: Blood Collection in Sheep

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 Treatment4
- VII. References5

I. Procedure Summary and Goal

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

The right and left external jugular veins are large superficial veins that lie within the jugular furrow, a groove on each side of the neck dorsolateral to the trachea.

Considerations:

- a. Sheep are generally docile in nature, and exhibit strong flocking behavior; they may become very anxious when separated from other sheep. Once caught, sheep typically will stand still.
 - i. Handlers should be vigilant at all times so as to avoid injury to animals or themselves.
 1. Head and/or horn butting
 2. Hoof – may strike or step on handler’s feet
- b. 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
 - ii. Sheep Estimated Circulating Blood Volume = 60ml/kg
 1. Example: 40kg sheep: $CBV = 20\text{kg} \times 60\text{ml/kg} = 1200\text{ml}$. Maximum one time survival withdrawal (without fluid supplementation) = $0.10 \times 1200 = 120\text{ml}$

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
 1. Disposable gloves (e.g., latex, nitrile)
 2. Eye protection
 - iv. 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 be disinfected between barns or housing areas.
- c. Promptly dispose of used sharps in the provided leak-proof, puncture resistant sharps container.

III. Supply List

- a. Blood collection vials (e.g., syringes, vacutainer tubes)
- b. Needles (18 gauge or smaller; 1 to 1.5 inch) or vacutainer needles and hub
- c. Restraint (e.g., halter, stocks, head catches)
- d. Clippers (optional)
- e. Antiseptic
- f. Gauze

IV. Detailed Procedure

- a. Restrain animal with head elevated and jugular vein exposed (Figure 1).
 - i. Stand sheep with animal's back against your legs. Alternatively, set the sheep on its rump with its back against your legs (tipping or "set-up"). Hold the head of the sheep at about a 30° angle to the side to extend neck and expose jugular.
 - ii. Collection is most easily performed with two handlers – one to restrain and one to collect blood. With experience, one handler can hold and collect blood – with sheep set between your legs, tuck head under arm, and access jugular from above.
- b. Clip (optional) a small area over the jugular groove, 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 at the base of the jugular groove and visualize raised vein.
- d. With bevel up, insert needle through skin and into vein at 20° angle (Figure 2).
- e. Using vacutainer method - 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 (Figure 3).
- f. Using needle and syringe method – before use, break the seal on the syringe by gently pulling back plunger. Clear air, and with needle attached to syringe, insert needle at 20° angle, and aspirate syringe to confirm insertion and collect blood.
- g. If you have missed the vein, you can carefully reposition needle until vessel penetrated. Vessel may be fairly deep and 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.

NOTE: When using vacutainer, do not pull needle out of skin with vacutainer tube attached, as this will cause vacuum to be lost.

- h. Once collection complete, release pressure to the vein (and detach vacutainer tube if used), then, applying pressure over injection site with gauze, remove needle.
- i. Dispose of needle in approved Sharps container.
- j. In order to ensure adequate hemostasis, apply pressure with gauze for 30 to 60 seconds.
- k. Serial samples can be taken by alternating sides, and by moving insertion sites caudally, as long as there is no hematoma formation.



Figure 1. Pull Head to the Side and Occlude Jugular to Visualize Vein



Figure 2. Insert Needle



Figure 3. Collect Blood

V. Variations

- a. Alternate collection sites in sheep
 - i. Cephalic vein, typically in "set-up" position
 - ii. Femoral vein, in lateral recumbency

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

VII. References

- Allen, M. and Borkowski, G. The Laboratory Small Ruminant. (CRC Press LLC, Boca Raton, FL 1999)
- American Association of Laboratory Animal Science. Assistant Laboratory Animal Technician Training Manual. (Drumwright and Co, Memphis, TN 2012)
- Donovan, J., and Brown, P. Removal of blood from laboratory mammals and birds: First report of the BVA/FRAME/RSPCA/UFAW Joint working group on refinement. Lab Animal 27, 1-22. (1993)
- Hawk, C.T., Leary, S.T., and Morris, T.H. Formulary for Laboratory Animals (3rd ed.). (Blackwell Publishing, Ames, Iowa 2005)
- Holtgrew-Bohling, K. Large Animal Clinical Procedures for Veterinary Technicians (2nd ed.). (Elsevier Mosby, St. Louis, MO 2012)
- McCurnin, D. and Bassert, J. Clinical Textbook for Veterinary Technicians (5th ed.). (Saunders. Elsevier, Philadelphia, PA 2002)