

BLOOD SAMPLE COLLECTION**1. PURPOSE**

To describe how to safely collect blood samples from cattle.

2. RESPONSIBILITY

2.1 Trained and Qualified Personnel

2.2 Veterinarian

*** NOTE**

- Demonstrator: The operators should be familiar with the correct techniques and the anatomy of cattle before attempting this procedure
- Students: The procedures must be clearly demonstrated before students attempt them. Students should be aware of the requirements for sterile technique.

3. MATERIAL

The type of material used will vary according to the collection method.

3.1 Disposable gloves

3.2 Halter

3.3 Clippers (optional)

3.4 Vacutainer collection:

3.4.1 20G1" vacutainer needle: Coccygeal vein collection

3.4.2 18G1.5" or 20G1.5" vacutainer needle: Jugular vein

3.4.3 One vacutainer tube or bottle per animal (lavender, green, red)

3.4.4 Vacutainer tube holder

3.5 Syringe Collection:

3.5.1 20G1" needle: Coccygeal venipuncture

3.5.2 16-18G2" needle: Jugular venipuncture

3.5.3 1 – 5 cc syringe

3.6 Bleeding Tube Collection (> 100 ml)

3.6.1 2 x 14G2" vacutainer needles

3.6.2 500ml heparinized bottle (for collection of large volume e.g. 500ml)

3.6.3 Heparin

3.6.4 Bleeding tube (for collection of large volume, e.g. 500ml)

3.7 Antiseptic or alcohol pads

3.8 Gauze

3.9 Sharps container

3.10 Biohazard box for syringe disposal

4. CONSIDERATIONS

- 4.1 Minimal restraint is usually required to obtain blood samples from a cow (ex. haltering and restraining her head). If restraint is necessary, it should be executed to ensure quick, easy, and safe collection of the sample causing minimal distress.
- 4.2 A maximum number of three venipunctures should be attempted per sampling site and time point.
- 4.3 For multiple samples to be collected over a short time frame (e.g., excess of 10 punctures in a 24-hour period), a butterfly needle, percutaneous (over the needle) catheter, or another type of catheter fixed in position should be utilized rather than repeated needle punctures.

TABLE.1: NC3Rs Blood Sample Volumes

Blood Volume	Total Blood volume (TBV), normal adult (ml)	Safe volume for single
60	27,000 - 36,000 (assumes adult weight 600-	2700 - 3600

TABLE 2: Limiting Volumes and Recovery Periods

% Circulatory blood volume removed	Approximate recovery period*
SINGLE SAMPLE	
7.5%	1 week
10%	3-4 weeks
MULTIPLE SAMPLES	
7.5%	1 week
10-15%	3-4 weeks

TABLE 3: Needle Size for Blood Sample Volume

Needle size	Volume
14G 2"	(>20 ml)
16- 18G 1.5"	(<20 ml)
20G 1.5"	(1-10 ml)

5. PROCEDURES

- 5.1 Ensure that the animal is properly restrained to avoid injury to the animal and/or study personnel.
- 5.2 This activity must be performed by qualified personnel and with the assistance of a handler. It may be performed alone ONLY under exceptional circumstances.
- 5.3 Use clean gloves to prevent the transmission of blood-borne diseases.
- 5.4 Equipment such as vacutainer holders must be cleaned between animals.
- 5.5 Use a fresh bleeding needle with every cow.
- 5.6 **JUGULAR VENIPUNCTURE USING VACUTAINER NEEDLE AND TUBES:** (<100 ml)
 - 5.6.1 Using the halter, position the animal's head so that it is slightly elevated and drawn to the side to expose the jugular vein. (Fig. 1) The collar can be removed if it impedes access to the sampling site.
 - 5.6.2 Screw the 18G1.5" or 20G1.5" vacutainer needle onto the Vacutainer holder.

- 5.6.3 Insert the vacutainer tube into the other end of the Vacutainer holder. DO NOT puncture the stopper.
- 5.6.4 Hold these assembled materials in one hand.
- 5.6.5 Disinfect the venipuncture site with alcohol.
- 5.6.6 Occlude the jugular vein by applying pressure in the jugular groove located in the lower neck. (Fig. 2)
- 5.6.7 Position the needle bevel-up and insert it into the distended jugular vein at a 45° angle cranial to the jugular groove. (Fig. 2)



FIGURE 1: Restrain the animal to expose the jugular



FIGURE 2: Occlude the vein and insert the needle.

- 5.6.8 Once the needle is positioned in the vein, insert a vacutainer tube into the needle to collect the blood.
- 5.6.9 When the desired volume has been collected (5 ml minimum suggested) remove the occluding pressure from the vein.
- 5.6.10 If collecting more than one sample (e.g., different tube types), leave the needle and vacutainer holder in situ and insert the next blood tube.
- 5.6.11 Once the sample has been collected, detach the tube from the needle and withdraw the needle from the jugular vein.

* NOTE

DO NOT retract the needle with the Vacutainer still attached. This will collapse the vein, release the vacuum from the Vacutainer, and possibly affect the quality of the sample.

- 5.6.12 Apply pressure with gauze for 30 to 60 seconds to ensure adequate hemostasis.
- 5.6.13 Invert the tube several times to mix.

5.7 JUGULAR VENIPUNCTURE USING SYRINGE AND NEEDLE: (<100 ml)

- 5.7.1 Attach the needle (Table 3) to the syringe, based on the volume of blood to be sampled. Refer to Table 3.
- 5.7.2 Using the halter, position the animal's head so that it is slightly elevated and drawn to the side to expose the jugular vein. (Fig.1)
- 5.7.3 Disinfect the venipuncture area with alcohol.
- 5.7.4 Occlude the jugular vein by applying pressure in the jugular groove located in the lower neck. (Fig.3)
- 5.7.5 Position the needle bevel-up and pass it through the skin and into the vein by a firm thrust at an angle of 20° to the skin surface. (Fig. 3)
- 5.7.6 Withdraw the blood sample.



FIGURE 4: Occlude the vein, insert needle, and withdraw

- 5.7.7 When the desired volume has been collected (5 ml minimum suggested), remove the occluding pressure from the vein. And remove the needle.
- 5.7.8 Apply pressure with gauze for 30 to 60 seconds to ensure adequate hemostasis.
- 5.7.9 Dispose of needle(s) in a Sharps container.
- 5.7.10 Transfer the blood to the appropriate vacutainer tube.
- 5.7.11 Invert the tube several times to mix.
- 5.7.12 Several samples can be taken by alternating sides, and by moving insertion sites cranially, if there is no hematoma formation. Refer to Table 2: Limiting Volumes and Recovery Periods.

5.8 JUGULAR VENIPUNCTURE USING BLEEDING TUBE (> 100 ml):

- 5.8.1 Flush the Bleeding tube with heparin to prevent coagulation during collection.
- 5.8.2 Attach a 14G 2" vacutainer needle on each end of the bleeding tube.
- 5.8.3 Disinfect the rubber seal of the heparinized bottle with alcohol.
- 5.8.4 Uncap the needle of one end of the bleeding tube and insert into the rubber top of the heparinized bottle.
- 5.8.5 Disinfect the venipuncture area with alcohol.
- 5.8.6 Uncap the needle on the other end of the tube and insert into the distended jugular vein bevel-up at a 45° angle cranial to the jugular groove. (see Figure 2 for positioning)



FIGURE 5: Attach a vacutainer needle to each end of the bleeding tube.

- 5.8.7 You can collect more than one bottle by transferring the needle on the bottle end to another bottle. Refer to Figure 1 NC3Rs Blood Sample Volumes for safe collections for a single bleed.
- 5.8.8 When the desired volume has been collected, detach the bleeding tube from the needle and withdraw the needle from the jugular vein.

DO NOT retract the needle with a vacuum-sealed collection bottle still attached. This will collapse the vein, release the vacuum from the bottle, and possibly affect the quality of the sample.

- 5.1.1 Invert the bottle several times to mix.

- 5.1.2 Apply pressure with gauze for 30 to 60 seconds to ensure adequate hemostasis.
- 5.1.3 If additional samples are required after the needle has been removed, they can be taken by either alternating sides, or by moving insertion sites cranially, as long as there is no hematoma formation.

5.2 COCCYGEAL VENIPUNCTURE USING VACUTAINER NEEDLE AND TUBES (<100 ml):

- 5.2.1 Restrain the cow to prevent her from moving away during the procedure.
- 5.2.2 Raise the tail vertically until it is horizontal with the ground and the ventral surface of the tail is accessible.
- 5.2.3 Locate the groove lying in the ventral midline of the tail. (~150 mm from the base of the tail). (Fig. 6)



FIGURE 6: Coccygeal Vein

- 5.2.4 Screw the 20G 1" Vacutainer needle onto the Vacutainer holder.
- 5.2.5 Insert the vacutainer tube into the other end of the Vacutainer holder. DO NOT puncture the stopper. Hold the assembled material in one hand.
- 5.2.6 Disinfect the venipuncture area with alcohol.
- 5.2.7 Midway along the body of a coccygeal vertebra, position the 20G 1" needle attached to the vacutainer holder bevel-up, and insert it perpendicularly (90°) to the surface of the skin to a depth of a few millimeters.
- 5.2.8 Once the needle is positioned, push the vacutainer tube up into the needle to collect the blood. If blood does not flow into the tube, reposition the needle by withdrawing it slightly.
- 5.2.9 Collect the desired volume (5 ml minimum suggested).
- 5.2.10 If collecting more than one sample (e.g., different tube types), leave the needle and vacutainer holder in situ and insert the next blood tube.
- 5.2.11 Once the sample has been collected, detach the tube from the needle and withdraw the needle from the jugular vein.

DO NOT retract the needle with the Vacutainer still attached. This will collapse the vein, release the vacuum from the Vacutainer, and possibly affect the quality of the sample.

- 5.2.12 Apply pressure with gauze for 30 to 60 seconds in order to ensure adequate hemostasis.
- 5.2.13 Invert the tube several times to mix.

5.3 COCCYGEAL VENIPUNCTURE USING SYRINGE AND NEEDLE (<100 ml):

- 5.3.1 Restrain the cow to prevent her from moving away during the procedure.
- 5.3.2 Raise the tail vertically until it is horizontal with the ground and the ventral surface of the tail is accessible.
- 5.3.3 Locate the groove lying in the ventral midline of the tail. (~150 mm from the base of the tail). (Figure 6)
- 5.3.4 Disinfect venipuncture area with alcohol.

- 5.3.5 Midway along the body of a coccygeal vertebra, insert the 20G 1" needle, bevel-up, perpendicularly (90°) to the surface of the skin to a depth of a few millimeters. (Figure 7)
- 5.3.6 Withdraw blood sample (Figure 8) and remove needle. To ensure adequate hemostasis, apply pressure with gauze for 30 to 60 seconds.
- 5.3.7 When the desired volume has been collected, remove the occluding pressure from the vein., and remove the needle.



FIGURE 7: Insert the needle in the groove lying in the middle of the tail.



FIGURE 8: Withdraw the blood sample.

- 5.3.8 Apply pressure with gauze for 30 to 60 seconds to ensure adequate hemostasis.
- 5.3.9 Transfer the blood to the appropriate vacutainer tube.
- 5.3.10 Invert the tube several times to mix.
- 5.4 Promptly dispose of used sharps in the sharps container. Note: Needle holders can be re-used.
- 5.5 If required, identify the tube(s) / bottle(s) with the animal's identification name or number and farm name and complete the sample form.
- 5.6 Place the bottles or tube(s) in the refrigerator until picked up. If shipping, wrap the bottles/tubes in bubble wrap and place them in an insulated box with ice packs.

6. REFERENCES

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