1. PURPOSE

Liver biopsy is a technique to collect liver cells for molecular and biochemical analyses. This enables repeated collection of liver samples from the 10th intercostal space.

2. RESPONSIBILITY

Principal Investigators (PI) and their research staff, farm staff, veterinary staff and all qualified personnel who perform or assist with Liver Biopsy in Cattle.

3. MATERIALS

3.1 Personnel Protective Equipment (coverall, latex/ nitrile gloves, long-sleeve plastic gloves, CSA-approved steel-tie boots, etc.)
3.2 Halter
3.3 Sterile Tru-Cut liver biopsy needles (14 gauge, 6 inch; Care Express Products Inc.)
3.4 Oxytetracycline (20 mg/kg)
3.5 Lidocaine HCl 2%
3.6 Chlorhexidine scrub
3.7 Isopropanol
3.8 Iodine spray
3.9 Blu-Kote® (Bahr Saddlery Ltd)
3.10 Scalpel blades
3.11 Tweezers
3.12 Collection tubes (15 ml and 50 ml)
3.13 Liquid nitrogen tank.

4. GENERAL

4.1 It has been experimentally demonstrated that multiple liver biopsies can be taken from the same cow without any risk to the cow’s health.6,1
4.2 Duration:
   4.2.1 5-10 min for site preparation
   4.2.2 1-2 min for biopsy

5. PROCEDURE

5.1 Restrain the animal in such a way that it cannot back up.
5.2 Identify the biopsy area by drawing an imaginary line from the hook bone to the elbow bone and where it intersects the 10th intercostal space, which is the third space from the last intercostal space (approximately 1/3rd from topline in the 10th intercostal space).
5.3 Shave an area of about 10X10 cm at the biopsy site.

5.4 Antiseptic skin preparation:

5.4.1 Use aseptic technique when performing skin antisepsis.

5.4.2 Start at the center of the surgical site and move to the outside of the prepared area in a circular manner.

5.4.3 Perform three scrubs with a non-soap solution (e.g., alcohol, diluted non-soap antiseptic or sterile water) and three alternating rinses with an antiseptic soap solution (e.g. 2% chlorhexidine solution or povidone-iodine solution).

5.5 Induce local anesthesia by injecting 3-5ml lidocaine into the intercostal muscle and under the skin in the prepared biopsy area.

5.6 Wait at least 5 minutes and make sure of the induction of anesthesia using gentle needle pricks in the area, which should not elicit twitching skin response. Repeat 5.5 if there is a response.

5.7 Using a scalpel blade, make a small stab incision (1-3 mm) for needle insertion. This optional step helps with easy insertion of the biopsy needle avoiding tough skin and the incision is so small that it does not need to be closed at the end of the procedure.

* PRECAUTION

Monitor the animal for signs of pain (e.g. hunching back, twitching of abdominal muscle, kicking, etc.). In such case, stop and reevaluate local anesthesia.

5.8 Biopsy:

5.8.1 Insert the sterile biopsy needle with the stylet withdrawn into the needle for about 12-14 cm. The needle should be parallel (or slightly downward angle) to the ground and slightly angled forward.

5.8.2 Advance the stylet into the liver without moving the needle. The notch in the stylet with liver tissue.

5.8.3 Advance the needle while holding the stylet stationary. **IT IS CRITICAL NOT TO PULL THE STYLET BACK BECAUSE THERE IS NO CUTTING SURFACE ON THE STYLET.** The sharp edge of needle point cuts the biopsy sample into the notch.
5.8.4 Advance the needle all the way over the stylet. This captures the liver sample in the notch.

5.8.5 Remove the needle with stylet and collect the liver biopsy with sterile tweezer/forceps. This procedure yields 15-20 mg of liver tissue with each biopsy.

5.8.6 Transfer in a study appropriate container, media and keep at required temperature for storage or analysis.

5.9 Spray the biopsy area with Blu-Kote® to ensure infection-free healing of the incision area.

5.10 Administer systemic antibiotics (Oxytetracycline (0.1 ml/kg) intramuscularly) to avoid infection. Document in the treatment log book and ensure prescribed milk and meat withdrawal periods are respected.

**Step 1:**

**Step 2:**

**Step 3:**

**Step 4:**

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6. REFERENCES

6.2 https://www.dcpah.msu.edu/sections/nutrition/WEBCD.NUTR.REF.002.pdf