SOP for Collection and transport of tissue from cattle sampling (University of Alberta, UAL) BovReg

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1. PURPOSE

1.1 The objective is to describe how the age of cattle carcasses are determined prior to SRM sampling, and outline practices to ensure the safe and timely collection of various tissues for subsequent DNA/RNA extraction. The storage of samples prior to transport is also described.

2. MATERIALS & EQUIPMENT

2.1 In order to accurately identify the age of the carcass, adequate record keeping materials are needed to confirm date of birth of the animal.

2.2 Non-SRM tissues will have designated tissue collection materials that will be noticeably labeled as non-SRM materials.

- 2.2.1 Disposal No. 23 Swan Morten scalpels, scalpel blades, sterile forceps, sterile scissors, sharps collection container
- 2.2.2 Autoclave bags, autoclave safe plastic containers, autoclave
- 2.2.3 70% EtOH, 2N NAOH solution, 10% bleach solution, sterile PBS, RNA Zap
- 2.2.4 Cutting boards, sterile stainless steel containers for tissue collection, paper towel
- 2.2.5 Sharps container, Rubbermaid containers with lids, large Ziploc bags, 50 mL conical tubes, 15 mL conical tubes, 1 oz. Whirl-Pak sampling bags, 2 mL cryovials
- 2.2.6 LN2, coolers, dry ice, wet ice, chemical waste bags, 2L Liquid Nitrogen dewars (1 per sample table)
- 2.2.7 Petri dishes, Cable ties, biopsy punch, disposable razors, tin tissue collection containers
- 2.2.8 1L sterile plastic container with lid, serum vacutainer tubes, Tempus tubes, EDTA vacutainer tubes, swing bucket centrifuge
- 2.2.9 Disposable gloves (S,M,L), disposable surgical gowns, steel toed rubber boots, face mask

2.3 Labeled coolers with dry ice are used to transport tissues; and snap frozen tissues are collected in 1 oz. Whirl-Pak bags.

3. DETAILED STEP-BY-STEP PROCEDURES

3.1 Animal age is confirmed using the animal ID and the date of birth data associated with the ID. Additionally, the gender of the animal is confirmed. All sample tissue is collected from the left organ where applicable, and one stock sample is collected from the right, where applicable.

3.2 All surfaces in contact with tissue disinfected with RNAZap, rinsed with sterile water, and sprayed with 70% EtOH.

- 3.2.1 Ensure cutting boards, and stainless steel trays are all cleaned and sterilized before placing tissue on their surfaces.
- 3.2.2 Once animal is cut open, organs are placed in stainless steel trays on ice at the sampling stations until ready to be sampled.

3.3 Each tissue has designated samplers with specific and detailed protocols to follow at sampling station.

- 3.3.1 Sampling station is set up with waste biohazard bag, sterile PBS, 70% EtOH. Kimwipes, Paper towel, and tray for wet ice.
- 3.3.2 Excess tissue is put in designated non-SRM tissue waste bins around facility for disposal
- 3.3.3 All collected tissue is placed in pre-labeled 1 oz. Whirl-Pak bag and directly into 2L liquid nitrogen (LN2) dewars to be snap frozen.

3.4 **Liver collection**: Liver is removed and caudal lobe is cut from the organ.

- 3.4.1 Lobe is rinsed with sterile PBS; sample is divided into cortex and medulla with a lateral slice

3.4.2 Two small (½ inch x ½ inch) samples are cut as proximal from gall bladder as possible, and an extra 1 inch x 1 inch piece as a stock sample.

3.5 Lung collection: Lungs are removed and rinsed thoroughly with sterile PBS.

3.5.1 Lobe is rinsed with sterile PBS; small piece (½ inch x ½ inch) is cute as proximal from gall bladder as possible

3.5.2 Slice sample to divide cortex and medulla, collect two samples and 1 stock.

3.6 Spleen Collection: Spleen is detached and removed from abdominal cavity and rinsed with sterile PBS.

3.6.1 Rounded edge not connected to cavity cut approximately 6 cm into organ. Ensure to avoid blood vessels, and cut around the round edge to expose middle pulp.

3.6.2 Using scissors, remove the outer sheath so the pulp is accessible, cut into two 1½ inch x 1½ inch samples, and a larger one for stock.

3.7 Kidney Collection: Kidneys with fat attached are collected from main cavity and rinsed with sterile PBS.

3.7.1 Fat samples are cut using a disposable scalpel from both left and right kidneys, and placed in the correctly labelled 1 oz. Whirl- Pack bag.

3.7.2 Left kidney lobe is cut in half and two ½ inch x ½ inch sections of medulla are taken for sampling.

3.7.3 Right kidney is cut laterally in half and sample of medulla taken as stock sample.

3.7.4 **Adrenal Collection**: Adrenals should also be collected with kidney sampling, and sampled in the same manner, without fat collection.

3.8 Pancreas Collection: Pancreas is removed from the digestive tract material, and rinsed with sterile PBS.

3.8.1 Pancreas is placed flat and small edge referred to as 'legs' is cut approximately 3 cm into organ.

3.8.2 Two ½ inch x ½ inch samples are cut and an extra for stock if available.

3.9 Heart Collection: Heart is removed intact and sliced laterally to expose heart wall and separate atrium and ventricle.

3.9.1 Rinse with sterile PBS to remove as much blood as possible over metal collection pan

3.9.2 Cut into heart wall from left side of heart and sample two ½ inch x ½ inch pieces, and an extra larger piece for stock.

3.10 Thyroid and Thymus Collection: Remove thyroid and thymus from neck of cattle, often connected with lungs, and rinse thoroughly with sterile PBS.

3.10.1 **Thyroid**: locate left and right lobe of thyroid gland, extract left lobe from trachea, ensuring not to collect muscle. Leave intact and try not to dissect, unless too large to fit in 1 oz. Whirl-Pak sample bag. Stock sample is taken from the right lobe.

3.10.2 **Thymus**: Locate upper and lower thymus tissue, collect two ½ inch x ½ samples from lower section, and a 1 inch x 1 inch sample as stock from the upper tissue.

3.11 Muscle collection: Muscle tissue to be collected from carcass after internal organs removed. Muscles are collected from left anterior leg and left rib of animal.

3.11.1 **M. Longissimus**: Count from the first rib to the 13th rub, and collect tissue from the space between the 13 and 14th rib, ensure to enter muscle from the inside of the body cavity.

3.11.2 Cut two 2 inch x 2 inch pieces, and an extra for stock from the same area.

3.11.3 **M. Semitendinosus**: Access left anterior leg and collect tissue approximately halfway down the back of the leg.

3.11.4 Cut two 2 inch x 2 inch pieces, and an extra for stock from the same leg.

3.12 Skin and Subcutaneous Fat Collection: These tissues can be collected at any time, but best after the internal organs have been removed.

3.12.1 **Skin**: Shave an area near the left hand side of the groin for the skin sample. After shaving, gently rinse with sterile PBS, and using a scalpel, cut a 5 inch x 5 inch

section, using a 10 mm biopsy punch separate subcutaneous fat, dermis and epidermis from one another.

3.12.2 Using a scalpel, sample two ½ inch x ½ inch and one extra for stock.

3.12.3 **Fat**: Subcutaneous fat can be collected at any time that is accessible, ensuring to collect from the same spot each time.

3.13 Reproductive organ Collection: Ovaries, mammary gland and uterine horn are sampled from female animals. The reproductive organs are removed prior to removing the main internal organs and directly after the digestive tract.

3.13.1 **Mammary Gland:** As best as possible, sample entire gland starting from the teat upwards towards the parenchyma to locate the lobes and ducts. Once gland is collected cut in half, sampling from a left teat, stock from the right.

3.13.2 **Uterine horn:** If corpus luteum is present, sample from that same uterine horn that produces the corpus luteum. Otherwise, sample from left uterine horn, with the stock sample from the right horn. Collect two ½ inch x ½ inch samples from the left horn, and one from the right.

3.13.3 **Ovaries:** Locate both ovaries, slice in half, and store each half in the same bag. Determine left from right after collection. If corpus luteum is evident, slice off and store in a separate bag to be snap frozen.

3.14 Blood Collection: Blood is collected immediately after bolting, from a jugular bleed into a sterile 1L bottle. Bottle can be filled to ensure there is enough blood for all of the subsequent samples.

3.14.1 **Tempus tubes**: These tubes are filled from the 1L bottle, and stored at RT for 30 mins prior to freezing on dry ice.

3.14.2 **EDTA tubes:** Three vacutainer tubes are filled with whole blood and, two of those are centrifuged at 1300xg for 10 minutes in a swing bucket centrifuge at 4°C. Remove plasma and buffy coat and place in 2mL cryovial and freeze on dry ice.

3.14.3 **Serum tubes**: after filling with blood, store at RT for 30 mins. After centrifuge at 1300xg for 10 minutes to collect serum, extract and put in 2mL cryovial tubes to freeze on dry ice.

3.15 Digestive Tract Collection: After blood collection

3.15.1 Tie off esophagus to rectum with twist ties (soaked in ethanol first) and carefully remove digestive tract, carry over/place on tray for sampling team.

3.15.2 Clamp off duodenum, cecum, jejunum and ileum and label these points.

3.15.3 Prior to removing connective tissue, locate caudal mesenteric lymph node and ensure not to discard. While removing connective tissue, compartmentalize the omasum, reticulum, abomasum and rumen.

3.15.4 Allow pancreas, liver and spleen (thymus, kidneys) to be removed by tissue runner and given to proper sampling team. After each section clamped/zip tied then cut each respective part.

3.15.5 Section should be 10-15 cm long, after tied off cut one end and squeeze contents into respective 50 mL tube for each section.

3.15.6 Duodenum sample collected 10 cm distal of pylorus- cut 2 large samples approximately 10-15 cm long , wash with PBS, store both as stock.

3.15.7 Jejunum collected at mid jejunum- also collect fat from this area, at the insertion point of jejunum- repeat method from 3.15.5 and 3.15.6.

3.15.8 Fat samples are collect as stock only, can collect other areas just record and re-label tubes.

3.15.9 Ileum samples are collected 10 cm distal of entry to colon/caecum, repeat method from 3.15.5.

3.15.10 Colon samples are collected 10 cm distal of the entry to the ileum, see sample method from 3.15.5.

3.15.11 Locate the caudal mesenteric lymph node and sample the most distal node, furthest from the abdomen. The reticulum, abomasum, omasum, and rumen, sample in a similar method as step 3.15.5, however record the areas chosen in the notes section. All samples stored in LN2 dewar found on table.

3.16 **Brain Tissue Collection:** After animal has been bolted, the head is removed and place in a vice to safely get access to the brain. Once the skull has been pried open, the full intact brain is removed.

3.16.1 **Frontal cortex**: Remove 2-3 cm of the most anterior portion of frontal cortex with SRM-designated knife. From this section, collect two small pieces (½ inch x ½ inch), and store a larger piece as stock.

3.16.2 **Cerebellum**: Cut from the rest of the brain with SRM-designated knife, and using a disposable scalpel take a slice from the middle section and cut into two 0.5 inch x 0.5 inch pieces, and larger for stock sampling. Follow the sampling images provided for areas of sampling.

3.16.3 **Hypothalamus**: Cut from the posterior behind the mammillary bodies, a few inches ahead of the optic chiasm. Make a lateral cut on the edge of optic chiasm (i.e. when they form the tractus optici (stripes), then cut at 2 cm depth to reduce the height of the block. This block is cut on its sagittal plan in 2 parts, the median eminence generally tears off, some may remain but its quantity cannot be assessed. Cut this section into two small ½ inch x ½ inch pieces and a larger as stock.

3.16.4 **Pituitary Gland**: Slice brain in half with SRM-designated knife; collect samples from osseus bulge harboring the gland. Collect two ½ inch x ½ inch samples, plus 1 stock**.**

3.16.5 **Olfactory Bulb:** Locate olfactory, collect intact left bulb, store the right bulb as stock.

3.17 Once tissues have been collected in pre-labeled 1 oz. Whirl-Pak bags, or 50 mL tubes, they are immediately snap frozen in liquid nitrogen.

3.18 The samples are then removed and placed into Ziploc bags, which also are labeled with the tissue contents, and placed on dry ice in designated Non-SRM coolers.

3.19 Coolers are sprayed with 70% ethanol before leaving sampling facility, and placed in the bed of a truck in a well-ventilated area to take back to the University of Alberta.

4 STORAGE OF SAMPLES

4.1 Once samples have been safely transported to the U of A, they are stored in labeled Ziploc bags at -80C.

5 ADDITIONAL CONSIDERATIONS

5.1 There are many sharp objects present at this sampling, sample blades are removed with disposal containers, and do not require individuals to physically touch the blade.

5.2 Individuals wear two pairs of gloves to ensure they do not come in direct contact with any fluid or tissue at any point.