Guidelines for Collection of Bird Tissues

All tissues should be stored in Nalgene cryogenic tubes. Tubes should be barcoded in the field. Contact a MVZ Staff Curator for both tubes and barcodes.

Liquid N_2 - High Quality Reference Genomes

- Different tissue types in individual tubes; if samples will be sent to an outside lab, take duplicates so that one set of tissues remain in MVZ:
    - Heart
    - Liver - take 2 tubes
    - Muscle - both white (breast) and brown (leg) muscle
    - Kidney
    - Spleen - whole spleen
    - Lung
    - Brain - make sure you do not just take the Medulla but also the hippocampus, brain stem etc. When opening the brain case in a traditional prep the Medulla is often pulled away with the cut back of the brain case, what remains contains the hippocampus and other brain regions. Place together in a tube.
    - Eye - place both eyes in a tube
    - Gonad (testes or ovary)
    - Tongue and palate - the palate needs to be cut from the roof of the back of the maximala.
    - Gizzard - take a piece of the gizzard wall; cut it open, wash away food content and then place in tube
    - Intestine - take 1 cm piece that leads from the gizzard toward the cloaca
    - Skin - if the bird is molting take a small piece of molting skin
    - Feather follicles - if the bird is moulting pull 3-4 growing feathers
    - If the bird has fat, collect some adipose tissue

Liquid N_2 - Standard Sampling

- Heart, liver, muscle, kidney stacked in tubes
- For malaria work, put tissues in this order: heart, kidney, muscle, liver

RNA L8R

- Time-sensitive (record approximate time from death to RNA L8R buffer): Put ~ 0.5mL of buffer in cryotubes
- Take the following tissues in separate tubes, noting time from death to tube:
    - **All individuals per species per site:**
        - Tube 1) liver + spleen → (label L + Sp)
        - Tube 2) heart + muscle + kidney → (label HMK)
    - **Up to 3 individuals per species per site:**
        - Tube 3) eye, brain, reproductive tissue → (label Eye, Br, testis/ovary)
        - Tube 4) lung + stomach wall + intestine with food removed → (label Lung + Guts)
        - Tube 5) blood → (label blood)
- General guidelines:
    - Cut open breast quickly and take muscle sample. Mince tissues to allow salts to permeate into the tissue faster.
    - For other tissues, cut down from breast and remove guts quickly
    - Be careful not to put too much tissue per tube, otherwise there will not be enough salts in the buffer to properly preserve the tissue.

- If LN_2 is available, drop tube into LN_2 after ~8 hours to allow time for the buffer to permeate throughout the tissue; otherwise, tubes will be fine at room temperature until they can be frozen.

DMSO Salt Buffer or 95% Ethanol:

- Put ~ 0.5mL of buffer or ethanol in non-cryotubes. Mince a small piece of muscle and put it into the tube. Again be careful to not put too much tissue per tube.
- Parasites: Collect endo- and ecto-parasites in 95% EtOH. Keep in separate tubes. → (label Endoparasite or Ectoparasite)
- Cloacal swabs: Use the appropriate swab size such that it can be inserted into the cloaca. Sterilize the area with diluted Hydrogen peroxide. Insert the swab into the cloaca and twist a couple of times to gather some of the cells lining the cloaca. Using a clean pair of scissors, trim the swab off into a cryotube filled with 95% ethanol. Fecal matter is on the swab OK but should be noted in your catalogue.
- Blood: Blood can be stored in a variety of solutions (e.g., Longmire buffer, 95% ethanol, etc). Alternatively, blood can be dabbed on sheets of Whatman filter paper and kept in a sealable plastic bag with desiccants.
- Syrinx: store in 95% ethanol.
- Tongue: store in 95% ethanol, include hyoid if possible.

Blood Slides

- Method 1:
  - Take out heart and rub thinly across slide w/o squeezing the heart
  - Label slide with collector # and species, in pencil
  - Let slides dry for ~ 5 minutes before putting them in the slide box
- Method 2:
  - 1. Wipe two slides clean with Kimwipes. This will facilitate step 4. Label one slide with the collector # and species in pencil, henceforth referred to as Slide A.
  - 2. Take blood using a capillary tube. Dab capillary tube on surface of Slide A about 1 cm away from white labeling area.
  - 3. Take Slide B and line up (without touching yet!) the edge opposite from the white labeling area with the blood from the capillary tube.
  - 4. Holding Slide B at a ~45-degree angle from Slide A, place the edge on the blood to spread the blood along the length of the slide, retract slightly towards the white label area, then quickly yet steadily push Slide B away from the white label area. Ideally this will form a smear with “feathering” at the end that is one cell thick. Slide B can be used as the next “Slide A” for the next sample.
  - 5. Let slide dry for ~ 5 minutes. Put into staining jar with methanol for at least 5-10 minutes.
  - 6. Let slide dry for ~5 minutes. Put slide into slide box.