

Necropsy Technique for Mammals Script

Intro

This video will show you how to conduct a thorough examination of a mammal carcass and how to collect vital samples that can aid in disease monitoring and detection.

Preparation

To collect a full set of samples, you will need:

- Personal protective equipment (or PPE), including an N95 respirator, eye protection, gloves, rubber boots, and protective clothing such as coveralls and/or an apron

- The proper forms on which to record your observations

- A camera to photograph your findings

- A ruler or measuring tape to measure abnormal findings and document scale in all photographs

- Permanent markers and a pencil for labeling

- Sample collection containers and supplies including 10% neutral buffered formalin, empty cryovials, cryovials of RNAlater and/or viral transport medium, and sterile swabs

- Tools such as scalpels and handles, scissors, forceps, saws, and knives.

Before you begin, make sure you have collected all relevant carcass information, including the collection location, environmental conditions, and circumstances of death, and that you have recorded this information on the appropriate forms.

Next, organize your team. If 2 people are available, designate one person as “contaminated” and one person as “clean”. The “contaminated” team member will handle the animal, make the cuts, and take samples. The “clean” team member will NOT handle the animal or any “contaminated” instruments or equipment. They will only handle the clean instruments, record observations, write labels and take pictures. This structure will help with biocontainment and minimize contamination during the necropsy.

Finally, prior to sampling, make sure to label all collection containers with appropriate identification information. All sample containers should include, at a minimum, an animal identification number, animal species, sample type, and date.

Necropsy

General:

At any time during the procedure, if you see something that you think might be abnormal, or if you are unsure of whether it is normal, take a picture and a sample of what you think is abnormal and adjacent normal tissue. Describe what you see in the necropsy form.

Pictures should include a tag with the date and the animal’s identification number and a section of measuring tape or a ruler for scale.

Describe observations using simple language including color, size, (length, width, thickness), and consistency. Remember that descriptions are not interpretations. Interpretations are based on all of the information and photographs that were gathered during the necropsy. Your partner veterinarian or pathologist can help with the interpretation of your findings.

Please note, when collecting samples into formalin, try to cut samples so they are not more than 1 cm thick and fill your container to have a 10:1 ratio of formalin to tissue. Failure to do so will interfere with proper tissue fixation and will compromise histologic examination.

External Exam:

Whenever possible, weigh the carcass.

Take measurements, including the total body length and length of the tail.

Assess and record the animal's sex, postmortem condition, body condition, and age class on the necropsy form.

Examine the carcass externally from head to toe. Note any wounds, fractures, parasites, or other external abnormalities. If you find any ectoparasites, place them in a cryovial with 70% alcohol.

Take photographs of the carcass and any abnormalities you may have noted.

Collect oropharyngeal and rectal swabs and place them in viral transport media and/or RNA later.

Large mammals should be positioned on their side and small mammals can be positioned on their back or side. When possible, place ruminants with the left side down due to the location of the rumen on the left side of the body.

With an animal on its side, reflect the top front leg by cutting the skin, subcutaneous tissue and muscle of the axilla down to the scapula. Open, disarticulate, and examine the shoulder joint. Reflect the top hind limb in the same manner as the forelimb cutting from the inguinal area deep to the hip joint. Open, disarticulate, and examine the hip joint. Extend the incision caudally along the midline. Separate the skin from the muscle without cutting into the body cavity.

If the animal is on its back, you will need to reflect both front and rear legs, open the joints as above, then make a midline incision and reflect the skin. Note the relative amount of subcutaneous fat that is present in the subcutis between the skin and the muscles.

Extend the midline incision along the underside of the neck up to the chin.

Collect a small piece of skin and muscle and place it in formalin. If genetic testing is of interest, place another sample in 70% ethanol.

On the hind limb, open, disarticulate, and examine the knee joints. Dissect through the muscle caudal to the femur, the bone above the knee, to find and collect the sciatic nerve. Place it in formalin. Next, collect a bone marrow sample from the femur. Crack the bone in two places, making sure to angle the cuts toward each other so that the bone splits. Place a sample in formalin.

CHECKPOINT 1

At this point you should have:

- Pictures of the carcass and any abnormalities that you found
- Notes, including the length and weight of the animal
- Rectal and oropharyngeal swabs
- Samples from the skin, muscle, sciatic nerve, and bone marrow.

Now you are ready to open the body cavities.

If the animal is on its side, make an L shaped incision to open the body cavity. Make the first cut from the sternum along the back edge of the ribs towards the spine. Make the second cut along the midline from the sternum towards the tail to the pelvis. Open the abdominal cavity by reflecting the abdominal wall. If the animal is on its back, make a T shaped incision to open the body cavity. Make the first cut from one side of the carcass to the other along the back edge of the ribs. Make a second cut along the midline from the sternum to the pelvis. Do your best to avoid cutting too deep and cutting the intestines or touching any organs prior to sampling them.

Note any fat stores and photograph the open body cavity before proceeding.

If any fluid is present, note its color, consistency, and measure or try to quantify its amount. Anything more than a very small amount of fluid is generally abnormal. Take a picture and a sample in an empty cryovial.

Note the size of the liver. Normal livers generally do not extend beyond the caudal edges of the ribs.

Now find the diaphragm. It is a curtain of muscle that separates the chest cavity from the abdominal cavity. Puncture it to assess the pressure in the chest cavity. If normal, it should collapse outward towards you. Look into the thoracic cavity. If any fluid is present, note its color, consistency, and estimate the volume. Collect a sterile sample using a sterile swab or syringe.

To continue opening the chest cavity, cut the diaphragm from one side of the body to the other along the back edge of the ribcage.

To remove the ribs, if the animal is on its side, cut the ribs parallel to the spine and along the midline. If the animal is on its back, cut the ribs along both sides of the body. Remove the rib cage.

Locate the heart and carefully cut open the sac around the heart to expose it.

Before touching anything, take pictures of the thorax and abdomen with the organs in the locations in which you found them. Note any abnormalities.

Before handling any tissues, collect sterile samples for molecular diagnostics, such as tissue samples in VTM or RNA later, or swabs for bacterial or mycotic cultures.

CHECKPOINT 2

At this point you should have:

Photographs of the thoracic and abdominal cavities in situ
Sterile tissue samples for molecular diagnostics or culture.

Now it's time to examine individual organs and organ systems. Starting with the chest cavity, examine and sample the thyroid gland. The thyroid glands are small pink to red organs located on both sides of the neck at the base of the thick ring of cartilage near the top of the trachea. Note any abnormalities and place the thyroid glands in formalin.

Now remove the 'pluck' – this is a set of tissues that includes the tongue, esophagus, trachea, heart, and lungs. Make an incision on either side of the tongue so you can push the tongue through the bottom of the jaw. Then pull the tongue towards you while you cut the connective tissues to remove the trachea, and esophagus along the length of the neck. Continue peeling the pluck towards the diaphragm. Remove the pluck from the chest cavity by cutting the esophagus at the level of the diaphragm.

Examine the external aspects of the tissues noting any abnormalities. Collect a full-thickness, cross-section sample of the tongue and place it in formalin. To examine the interior of the trachea and esophagus, cut open the trachea along its long axis, noting any fluid and take a picture. Make a similar longitudinal cut down the esophagus. Collect a cross-section sample of the trachea and esophagus together and place it in formalin.

Remove the heart. Note that the heart has a right side, left side and center section. The right side is smaller and thinner than the left. Each side has a top (atrium) and bottom (ventricle) section. Open the right and left sides of the heart. Collect longitudinal samples from the top to the bottom of the heart that include the atrium and ventricle from the right and left sides. Collect a similar top to bottom sample from the center section of the heart in formalin.

Lay the lungs out and photograph them.

Then make evenly spaced parallel cuts across it to examine the tissue. This is called bread-loading, like slicing a loaf of bread. Note any liquid that oozes out when you cut them. Photograph the lungs cut into sections and place at least one piece from each lung lobe in an empty cryovial and in formalin. Collect additional samples if there are any abnormalities. Note if the lung samples float or sink in formalin.

CHECKPOINT 3

At this point you should have:

- Pictures of the pluck in situ and uncut section.
- Pictures and notes of any abnormalities that you found.
- Samples from the tongue, trachea, esophagus, heart, lungs, and any abnormal tissue.

Moving into the abdominal cavity, locate the liver. It is a large dark red/brown organ sitting just behind the diaphragm. Remove the liver and take a picture of it whole. Breadloaf the liver to examine the tissue. Note any abnormalities and take a picture of the bread-loafed tissue. Place

a sample in an empty cryovial and place 2 to 3 pieces in formalin. If a toxin is suspected, collect a large piece of liver (~50g) in a plastic bag.

Now find the spleen. It is a long, thin, dark red to purple organ on the left side of the abdomen. Detach the spleen, photograph it, bread-loaf it, and collect samples in formalin and an empty cryovial.

To examine the digestive tract, urinary tract, and reproductive tract, remove the gastrointestinal tract en bloc from the diaphragm to the anus. Set it aside for examination later in the procedure.

Next find the urinary tract, gonads, and adrenal glands. The kidneys are dark red/brown bean-shaped organs near the spine.

The adrenal glands lie at the top end of each kidney. Photograph the kidneys with the adrenals in situ. Collect both adrenal glands into formalin.

Remove the kidneys. Cut the kidney in half along its long axis. Examine and note any abnormalities. Note that each kidney is covered by a thin capsule. Note its color and remove the capsule. Collect a full-thickness center section of each kidney into formalin and a smaller sample in an empty cryovial. If a toxin is suspected, place the kidneys in a plastic bag.

Examine the urinary bladder, ureters, and urethra. Place a sample from the bladder in formalin.

Examine the reproductive tract. A male will have two testes and accessory glands, such as the prostate gland. Females will have two ovaries and a uterus. Photograph the gonads. Make sure to open the tunic of the testes and subsection the testes and ovaries if they are large. Place samples of the gonads, accessory glands, and uterus in formalin.

CHECKPOINT 4

At this point you should have:

- Pictures of the liver, spleen, urinary tract, and reproductive tract.

- Pictures and notes on any abnormalities that you found, including any fluids.

- Samples of the liver, spleen, kidney, bladder, adrenal glands, gonads, and uterus.

Next, return to the gastrointestinal tract and linearize the tract on a clean surface. Lay it out flat and take a photograph.

Open the tract by cutting it longitudinally along its long axis from the esophagus to the small intestine. Note that depending on the species, the stomach may have one or multiple sections, such as in ruminants. Once opened, examine the contents of each stomach section. Remove the content and examine the inner surface of the stomach. Collect the contents of the stomach, or the abomasum in ruminants, into a plastic bag if a toxin is suspected. Take a full-thickness sample of all sections of the stomach.

Now open the intestine longitudinally.

Lay the opened intestine out flat on the clean surface and take a picture of it.

Note the appearance and amount of intestinal material and collect feces in an empty cryovial. If you find any endoparasites, place them in a cryovial with 70% alcohol. Collect a full-thickness, transverse sample of the duodenum with pancreas, jejunum, ileum with cecum, colon, and rectum in formalin.

CHECKPOINT 5

At this point you should have:

- External and internal pictures of the gastrointestinal tract and its contents.

- Pictures and notes of any abnormalities that you found.

- A fecal sample.

- Samples of the stomach, duodenum with pancreas, jejunum, ileum with cecum, colon, and rectum.

Next, examine the head. Remove a whole eye, by cutting the attachments between the eye and the orbital socket. Take a picture and place the eye in formalin.

Finally, examine and collect the brain. Remove the head by cutting through the joint joining the head and neck. When the head is free, remove the skin and muscle to expose the skull.

Cut the skull with bone cutters, an ax or saw, as appropriate for the head size.

Carefully remove the skull cap so you don't touch the brain. Take a picture of the brain in the skull and place a sample into an empty cryovial.

Remove the brain by cutting the connective tissues and nerves that anchor it within the skull. Take a photo from the top and bottom of the brain.

The pituitary gland sits in a shallow depression roughly in the center of the skull beneath the brain. Remove it and place a sample in formalin. If a toxin is suspected, place ~5-10g of the brain in a plastic bag. Place multiple sections of the brain or the whole brain if it's small in formalin.

CHECKPOINT 6

At this point you should have:

- A picture of the eye and brain.

- Photographs and notes on any abnormalities that you have found.

- Samples from the eye, brain, and pituitary gland.

You are now finished collecting samples!

Make sure all of your paperwork and samples are clearly and permanently labeled.

Clean up your work area and properly dispose of the carcass and of other necropsy materials.

Clean your equipment & containers thoroughly. Store samples frozen, on ice or in a refrigerator and ship samples for testing as soon as possible. Remember to submit the sample checklists, pictures, and forms as well.

Thank you for your help in monitoring wildlife disease. Every carcass counts!