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FOCAL POINT

- ★ A systematic necropsy helps ensure that each body system suspected to be involved in illness or death will be examined effectively and that appropriate samples are collected.

KEY FACTS

- Necropsy should be performed as soon after death as possible or within 24 hours if the body has been refrigerated.
- Microbiologic specimens should be generous in size or volume and placed in commercial transport containers.
- Samples taken for histopathology should be no thicker than $\frac{3}{8}$ inch and preserved at a 10:1 volume ratio of 10% buffered formalin: tissue.
- Bone marrow evaluation should be conducted using touch impressions taken as soon after death as possible.
- Historical, clinical, laboratory, and necropsy findings are essential for interpretation of samples and should accompany specimens submitted for diagnosis.

A Practitioner's Guide to Necropsy

Auburn University

Eva A. Sartin, DVM, PhD

Joseph S. Spano, DVM, PhD

Terri L. Hathcock, BS, MS

ABSTRACT: A well-performed necropsy can provide useful information to veterinarians and pet owners. This article provides an overview of the necropsy examination procedure using an organ system approach, which allows for variation depending on the clinical signs seen in the live animal and lesions observed during the necropsy. Proper specimen collection for microbiology, cytology, and histopathology is emphasized.

Necropsies frequently answer many questions that veterinarians and pet owners have concerning an animal's illness or death, such as the cause, reason for the lack of response to therapy, and legal issues. In addition, knowledge gained from a necropsy performed on an animal with a poorly defined clinical illness may offer helpful insight into the treatment of other animals with similar conditions.

This article provides an overview of a systematic necropsy technique for small animals, with emphasis on sample collection for further diagnostic evaluation. Specific comments on some gross changes are made to help direct practitioners in the examination process. Excellent detailed information on normal and abnormal tissue characteristics, necropsy technique, and indications for sample collection is available in the literature.¹⁻⁴

GETTING STARTED

It is best to perform a necropsy immediately after death. When this is not possible, the body must be refrigerated immediately after death and the necropsy performed within 24 hours. If refrigeration is not available, euthanasia can be planned for a time when necropsy can be performed without delay. Otherwise, tissues will autolyze and become difficult or impossible to evaluate.

Before beginning the necropsy, practitioners must first anticipate their needs for the procedure and collect the appropriate materials. The safety of veterinarians and live patients is important, so common sense must be used; gloves and protective eye wear and clothing are needed. Instruments for the gross examination include a knife or scalpel, scissors, forceps, and bone cutters. Other requirements include specimen transport tubes and sterile instruments (for microbiology samples), clean glass slides and EDTA tubes (for cytologic evaluation), and a large-mouth container of 10% buffered formalin (for histopathology samples).

SAMPLE COLLECTION AND PREPARATION

The results of laboratory examination of specimens depend on the quality of

submitted specimens; proper technique is therefore vital. Using collection techniques that minimize contamination of microbiology samples improves the quality of the results. Urine, fluids, and aspirates can be collected using a syringe and needle. Microbiologic specimens should be generous in size or volume and placed in commercial transport containers. The buffering agents in the transport media preserve the integrity of specimens and survival of organisms without contaminating overgrowth. Samples should be transported immediately after collection; however, when a delay is unavoidable, the samples can be refrigerated at 4°C. If shipping is necessary, overnight delivery should be used and cool packs added to the shipping container.

Cytologic evaluation of some necropsy specimens can be an important adjunct to postmortem diagnosis. Liver, kidney, lymph node, spleen, and bone marrow are a few tissues that can be easily sampled for cytologic evaluation and provide useful, sometimes diagnostically decisive, information. The freshly cut surface of the tissue being sampled should be lightly blotted with a paper towel or gauze sponge to remove excess fluids. A clean slide should then be gently touched to the tissue's surface, lifted straight up (avoiding side-to-side movement), and quickly air-dried. Body cavity effusions, synovial fluid, and cerebrospinal fluid collected at necropsy can also provide important diagnostic clues. A fluid sample should be collected in an anticoagulant (preferably EDTA) for routine cell count, protein determination, and differentiation of cell types. Slides must not be exposed to formalin fumes because they greatly impair staining quality. Any commercially available quick stain can be used. Details of cytologic examination and diagnosis have been reviewed elsewhere.²

Histopathology samples should be no thicker than $\frac{3}{8}$ inch and preserved in 10% buffered formalin at a formalin:tissue volume ratio of 10:1. After 2 to 3 days, samples fixed at this ratio can be placed in a smaller volume of formalin for shipment. Frozen specimens are not suitable for histologic examination.

NECROPSY PROCEDURE

Like the clinical workup, the necropsy evaluation includes a general examination with emphasis on organ systems that have been noted to be abnormal. Unexpected findings discovered during necropsy may change the original focus of the examination. The examination begins by noting the animal's body condition, skin abnormalities, discharges, diarrhea, discoloration, nodules, masses, or anomalies.

Integumentary System

Samples of cutaneous lesions that include adjacent

normal skin should be collected for histopathology. When multiple lesions are present, samples of early-stage lesions as well as progressing lesions should be obtained. More information is obtained by examining early and progressing lesions than those that are chronic and healing. All mammary glands and the lymph nodes that drain them should be examined and then sectioned to reveal masses or other abnormalities.

Reflecting Limbs and Skin

Necropsies can be performed with animals in dorsal or lateral recumbency. Lateral recumbency is easier for large, deep-chested dogs. Limbs can be reflected by incising the skin and soft tissues at the axillae and coxofemoral joints of the uppermost limbs (for animals in lateral recumbency) or of all four limbs (for animals in dorsal recumbency). Skin should be reflected from the abdomen and thorax.

Peritoneal Cavity

The abdomen should be opened prior to the thorax to check the position of the diaphragm before releasing the negative pressure of the thorax. The abdomen is opened from the xiphoid to the pubis and the abdominal wall reflected to obtain maximum exposure of its contents. The color, consistency, transparency, and odor of the peritoneal fluid should be noted. Peritoneal fluid abnormalities may result from peritonitis, ruptured visceral or vascular structure, or neoplasia. This determination can be aided by microbiologic and cytologic examination of fluid (see Sample Collection and Preparation section). Samples for creatinine analysis of peritoneal fluid and blood may also be helpful. After fluid samples are obtained, organs should be checked for displacement.

Abdominal and Pelvic Viscera

The position of the diaphragm must be checked before removing the abdominal viscera. A diaphragm that is not convex indicates there is loss of negative thoracic pressure, a space-occupying lesion, or increased thoracic fluid. As the diaphragm is punctured, an inrush of air occurs as negative pressure is lost and the diaphragm relaxes.

Before removing the gastrointestinal tract, the patency of the bile duct should be checked by squeezing the gallbladder and observing the duodenum for filling. The bile duct can then be transected and the duodenum freed from the liver. Removing the gastrointestinal viscera involves transecting the abdominal esophagus, freeing the stomach and spleen from their attachments, and removing the small and large intestine from the mesentery. The distal colon can then be transected and

the entire intestinal tract removed. If a pelvic cavity lesion is suspected, the pelvic floor can be removed by cutting through the bone on each side of the pelvic symphysis. The colon and urogenital tract can be freed from the pelvic cavity by cutting the soft tissue around the anus and external genitalia. This approach is helpful when evaluating intestinal, reproductive, and lower urinary tracts. The adrenals should be removed and carefully sectioned to determine the cortical:medullary ratio (which should be approximately 2:1). The adrenals must then be immediately placed into formalin. The liver, kidneys, and diaphragm can then be removed.

Oral, Cervical, and Thoracic Viscera

The first step in examination of the thoracic cavity is to remove the ribs and take samples of pleural fluid for culture and/or cytology if indicated. Next, the skin should be reflected laterally from the mandibular symphysis to the thoracic inlet and the ventral cervical musculature removed to expose the thyroid and parathyroid glands. After these glands have been removed, they should be placed into fixative without sectioning, unless they are greatly enlarged. The tongue should be freed

from the mandible and the hyoid bones and soft palate cut to free the larynx. The oral, cervical, and thoracic viscera can then be easily removed together.

Respiratory System

The nasal cavities, larynx, external surfaces of the lungs, and mediastinum should be examined for nodules, masses, and pulmonary consolidation. The lungs must be palpated gently because just one well-intentioned squeeze can induce many artifacts. The larynx, trachea, bronchi, and smaller airways should be opened from proximal to distal. The content of the airway lumens should be examined for fluid and exudate before rinsing with water. Color change, which is a common postmortem change in lung tissue, must correlate with palpable abnormalities to be considered significant. Opened airways and vessels should be examined for exudate, thrombi, and/or parasites. Samples containing lesions should also include normal tissue.

Cardiovascular System

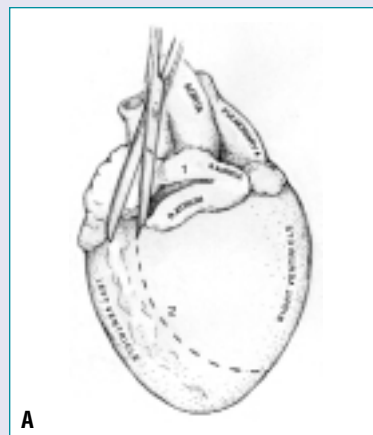
After death, the lower portions of the body (i.e., the "down side") will become darker (hypostatic congestion)

Opening the Heart

- Open the right atrium (RA) completely with scissors.
- **(A)** Open the right ventricle (RV) by placing scissors through the atrioventricular (AV) valve next to the interventricular septum and cutting the free wall of the RV adjacent to the septum, following a line from the AV valve to the apex.
- **(B)** Continue opening the RV by cutting the free wall of the RV along the septum through the pulmonic valve and well into the pulmonary artery.
- Open the left atrium. **(B)** Place scissors into this opening, through the left AV valve, and into the left ventricle (LV).

Balance the convexity of the LV across the blade, and cut to open the entire LV. To examine the aortic valve, cut through the septal leaflet of the left AV valve.

- Gently rinse away blood to inspect the chambers, valves, and vessels. Check endocardial surfaces, valves, and vessels for abnormalities. Make several sections into the myocardium, and look for areas of discoloration or masses. The LV:RV wall-thickness ratio should be approximately 3:1 in adults and 1:1 in neonates.



Figures A and B—The heart should be opened along the dotted lines in the right side of the heart (as shown in **A**), starting with the right atrium (1) and continuing into the right ventricle (2) and (as shown in **B**) beginning the pulmonary artery (3). The left heart can then be opened along the dotted lines (as shown in **B**) in the left atrium and continuing into the left ventricle (4).

than the upper portions. Clinical findings and lesions (e.g., petechiae, ecchymoses, severe hemorrhage, edema, icterus) can be used as clues for focusing dissections. Clinicians should look for evidence of thrombi or hemorrhage associated with vessels they transect.

The pericardial sac should be examined, the amount and characteristics of its content noted, and samples collected for culture or cytology if indicated. The inner surface of the pericardium should be examined and the pericardial sac removed, noting the shape of the heart and examining the epicardial surface. The heart can be opened following the path of normal blood flow (see Opening the Heart).

Digestive System

The oral cavity and external surfaces of the alimentary tract should be examined before opening the esophagus, stomach (along the greater curvature), and entire intestine. Microbiologic fecal specimens can be collected at this time and placed in a transport container specific for stool specimens. The mucosa should be rinsed gently with a stream of water because spraying or rubbing the mucosa will cause loss of mucosa and possibly a significant lesion. Samples for histopathology are obtained with forceps and scissors (Figure 1), again taking care not to rub the mucosa. Normal tissue should be included with the abnormal samples.

The liver should be examined for scars or nodules. Nodules could be abscesses, granulomas, hyperplasia, or metastatic neoplasia, and thus samples for culture, cytology, and histopathology should be collected if indicated. Because diffuse liver lesions may be subtle, samples for histopathology should be obtained even if lesions are not visible. The next step is to open and examine the gallbladder and bile duct. The pancreas should be examined for evidence of hemorrhage or inflammation. Small (i.e., 2 to 4 mm), sharply defined, white to yellow foci in the peripan-

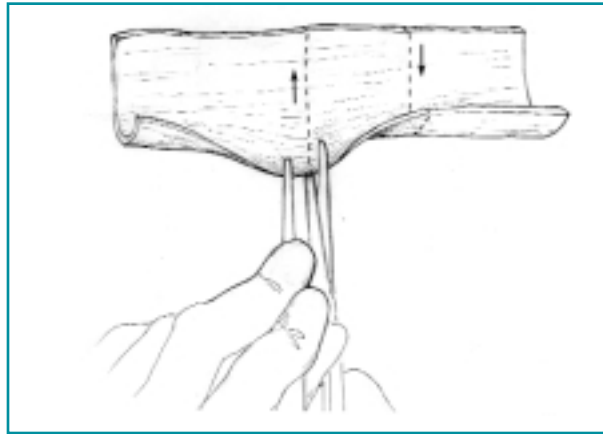


Figure 1—Obtaining a sample of a tubular organ without damaging the mucosal surface. Note that the forceps do not come into contact with the mucosa of the sample to be submitted (i.e., between the *dotted lines*).

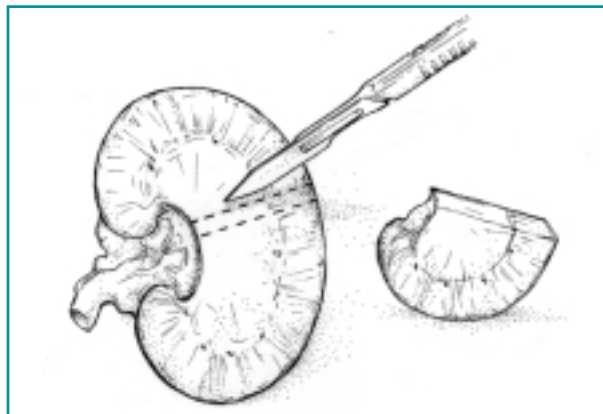


Figure 2—Obtaining a kidney sample that contains all parts of the nephron: The sample should extend from the outer cortex to the pelvis.

creatic and mesenteric fat are characteristic of fat necrosis, as is seen with pancreatitis.

Urinary System

The length of the kidney should approximate the length of three vertebrae. It should be sectioned midsagittally and the cut surface examined (the normal cortex:medullary ratio in the sagittal plane is approximately 1:2 to 1:3). The capsule is removed and the subcapsular surface inspected for depressions/scars, nodules, petechiae, abscesses, infarcts, or other lesions. Samples should be collected for culture or cytology if indicated. Samples for histopathology should extend from the outer cortex to the pelvis (Figure 2).

The ureters, urinary bladder, and urethra should be examined externally and then the urinary bladder partially opened to note the characteristics of the urine. Samples for culture and/or cytology should be taken if indicated. The opening in the urinary bladder can then be enlarged, extending from the fundus through the neck and into the urethra (including the prostatic urethra in males; Figure 3). Bladder tissue samples are obtained for histopathology as shown in Figure 1. If there is evidence of obstruction, the entire urethra and ureters should be opened.

Genital System

The external examination should reveal any abnormalities of the genitalia. The size and external and cut surfaces of the gonads of sexually intact animals should be examined for cysts, nodules, exudate, and scars. In females, the reproductive tract should be opened and any samples needed for culture or cytology collected before the mucosa is examined. Samples for histopathology should be obtained as shown in Figure 1. In males, the size, shape, color, and consistency of the prostate must be noted. The prostate can be sectioned

as shown in Figure 3 and checked for masses, discoloration, or exudate.

Hematopoietic and Lymphatic Systems

Cytologic examination of bone marrow specimens can provide important clues in determining the cause of hematologic abnormalities. Samples collected as soon after death as possible are of the greatest diagnostic value. A bone marrow sample can be obtained by cracking open a long bone, scooping the marrow from the extremities of the shaft, and making touch impressions. Interpretation of stained bone marrow specimens is reviewed elsewhere.² A second sample of bone marrow can be placed in formalin for histopathology.

Lymph nodes should be examined as they are exposed during the necropsy procedure. Enlarged nodes and those draining sites of inflammation or neoplasia should be sectioned to check the cortical and medullary architecture. The spleen and thymus are examined by making multiple sections into the splenic and thymic parenchyma and looking for abnormalities. Histopathology and cytology samples of lymph node, thymus, or spleen should be taken from areas that are free of excessive blood or necrosis.

Endocrine System

Thyroids, parathyroids, and adrenals are examined when the viscera are removed. If the adrenal cortex:medullary ratio is not approximately 2:1, then the pituitary should be examined following removal of the brain. The pancreas and adjacent mesenteric adipose tissue should be examined for evidence of hypoplasia, neoplasia, or inflammation.

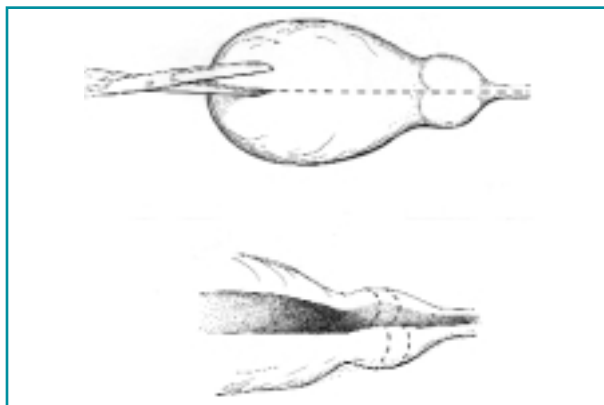


Figure 3—Opening the urinary bladder and prostatic urethra. (Top) Note that the incision in the bladder extends from the bladder to the prostatic urethra. (Bottom) Prostatic samples taken along the double row of dotted lines will allow histologic examination of urethral mucosa in the prostate.



Figure 4—Opening the calvarium to remove the brain. Bilateral cuts begin at the foramen magnum and dorsomedial to each occipital condyle and extend to just behind the orbits. A third cut that joins the two previous cuts is then made.

Musculoskeletal System

Joints should be opened by carefully incising the capsule to avoid contamination. If there are abnormalities in the synovial fluid or membrane, samples for culture, cytology, and histopathology should be obtained as indicated. The articular cartilage should be examined for discoloration and irregularities.

Determining which portions of bone to select when sampling bone lesions can be difficult. If possible, the entire bone—or at least a portion containing the lesion with adjacent normal bone—should be collected for histopathology. Areas of necrosis and periosteal proliferation may complicate sample selection, so the sample must consist of viable tissue and extend deep into subcortical bone and marrow.

Breaking strength and degree of calcification can be tested by isolating and bending a rib. Ribs that bend easily indicate poor calcification and suggest dietary mineral imbalance, severe renal disease, or parathyroid disease. When generalized bone disease is suspected, samples of flat and long bones should be taken.

If there is clinical evidence of generalized muscle disease, random incisions in various muscle groups, including the diaphragm, tongue, and heart, should be made to look for abnormalities. Multiple samples should be taken from various sites, even if they appear normal. Samples of focal lesions should include normal tissue.

Eyes and Nervous System

The eyes should be removed and peripheral nerves examined if indicated by clinical findings. Eyes are removed

by routine enucleation. The extraocular muscles should be trimmed carefully, without puncturing the globe, and the globe placed in fixative intact. Peripheral nerves can be examined as they are exposed during necropsy. Clinical findings will determine the extent of the peripheral nerve examination. If nerves contain or are associated with masses or nodules, samples should be obtained for histopathology.

If indicated by the history, the brain and spinal cord are removed. The brain is easier to remove if the head is disarticulated at the atlanto-occipital joint. The temporal muscles can be removed and the cranium opened as shown in Figure 4. Bilateral cuts are made through the cranium, beginning at the foramen magnum on the dorsomedial aspect of each occipital condyle and extending to just posterior to the orbits in the cranium. One cut is then made into the cranium just behind the orbits, joining the two previous cuts. The detached portion of the calvarium should be pried upward and carefully removed.



A Look Back

Although the necropsy procedure has several variations, the basic techniques have not changed significantly over the past 20 years. What have changed are the diagnostic tools that can be applied to collected specimens.

Advances in immunohistochemistry and molecular technology (e.g., polymerase chain reaction, in situ hybridization) have empowered veterinarians to make great strides in diagnostic interpretation of specimens and in understanding the pathogenesis for many infectious, neoplastic, metabolic, and genetic disorders.



The overlying dura and tentorium cerebelli are then gently removed. The head should be held upside down while the cranial nerves are transected from caudal to rostral. The brain should be allowed to fall into the clinician's (or assistant's) hand as its weight and gentle pressure pull it downward.

The pituitary should be removed if there is an adrenocortical abnormality or clinical evidence of pituitary dysfunction. This can be done by cutting through its dural attachment to the floor of the cranial cavity. The pituitary is very friable and thus must be immediately placed in fixative.

If rabies is a diagnostic differential along with several other neurologic conditions, appropriate precautions must be taken. The brain should be sectioned sagittally down the midline and half of it submitted for rabies evaluation; the other half is fixed in formalin for histopathology. If rabies is not suspected, the entire unsectioned brain should

be placed in formalin.

If the animal had clinical signs of spinal cord disease, the epaxial muscles and dorsal vertebral arches should be removed as is done with a dorsal laminectomy. The spinal cord should be carefully removed by gently grasping the dura with forceps, lifting slightly, and transecting each spinal nerve root as progress is made from cranial to caudal. Care must be taken to not bend the spinal cord sharply during removal because this will induce artifacts. The entire spinal cord should be placed in formalin.

COMMENTS

Samples that are easily folded and placed into a container with a small opening become hardened with fixation and can be very difficult to remove. In some cases, the sample may be damaged or destroyed when its extraction from the container is difficult. With this in mind, specimens should be placed in containers with wide openings. Questions about sample submission should be directed to the laboratory that will be receiving the specimens. A good history with accurate descriptions of gross lesions is an essential part of sample submission to a laboratory. Even if data are limited, this information significantly aids diagnostic examination and interpretation.

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About the Authors

Drs. Sartin and Spano and Ms. Hathcock are affiliated with the Department of Pathobiology, College of Veterinary Medicine, Auburn University, Alabama. Drs. Sartin and Spano are Diplomates of the American College of Veterinary Pathologists.