

Necropsy Techniques

General Pathology (VPM 152)

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"Physicians who either performed many autopsies themselves or who regularly witnessed post mortem examinations, learnt at least to have their doubts. Those, however, who are not themselves dealing with the very often depressing findings of autopsy material are floating in the clouds of uncontrolled optimism." Morgagni De Sedibus, 1761

"The Necropsy"

Necropsy Objectives

1. Expose all foci of disease/abnormality.
2. Seek lesions to explain clinical and laboratory findings.
3. Identify the sequence of disease events.

A conscious investigation, not a routine chore:

1. Systemic observation and dissection.
2. Collection and preservation of appropriate samples (tissue, fluids, etc) for histologic, cytologic, microbiologic, serologic, chemical, toxicologic, parasitologic, and/or radiologic evaluation.
3. Record findings logically, accurately, and completely.
4. Interpret findings:
 - a) immediate cause of death.
 - b) contributory causes.
 - c) other findings of clinical importance.
 - d) incidental findings.

Necropsy Equipment - will vary with the species, location of cadaver, etc.

CLOTHING: gloves, boots, coveralls, apron

INSTRUMENTS: sharp knife and sharpening equip (steel/stone)
tissue forceps and scissors
saw, cleaver, osteotome, shears, axe,
metric ruler, scale
soap, water, brushes for cleaning

FIXATIVES & MISC: fixative and appropriate containers
sterile syringes, needles, swabs, plastic bags, paper plates,
microscope slides, tags, dissecting microscope, photographic
capability (not essential)

STANDARD NECROPSY PROCEDURE

NOTE: procedural detail may vary, but a consistent technique aids in a thorough observation. To the beginner, necropsy techniques appear unreasonably cumbersome and regimented. The purpose is to methodically expose all organs and tissues to minimize the chance of missing or "destroying" a lesion.

PRENECROPSY EVALUATION:

1. identify the animal to ensure that the correct animal is being necropsied.
2. read the clinical history carefully.
3. examine the necropsy request form for the following:
 - a. special organs or systems clinicians may want examined.
 - b. whether or not this is a cosmetic necropsy.
 - c. special requests (ie, cultures, photos, etc).
4. fill container 75% full with 10% phosphate buffered formalin.
5. label container with necropsy number, species and initials of pathologist on duty.
6. weigh the animal - if possible.

NECROPSY EVALUATION

1. EXTERNAL EXAMINATION:

- a) note any abnormal external findings, egs;
 - Body Condition:** muscle mass / fat stores, decomposition, rigor mortis.
 - Skin and hair coat:** parasites, dehydration, tumours, wounds, scars.
 - Discharges from body orifices:** hemorrhage, nasal exudate, diarrhetic feces.
 - Eyes:** corneal opacities, unequal dilated pupils, exudates, ulcers, hemorrhages.
 - Ears:** parasites, tumours, discharges.
 - Mucous membranes:** colour, ulcers.
- b) take appropriate samples for culture, histology, cytology, etc.

2. POSITIONING AND OPENING THE CARCASS:

[may vary from pathologist to pathologist and from animal to animal]

- a) position the animal on its left side down.
- b) reflect the left front and left rear legs.
 - to save the cutting edge of knife, insert knife through skin and cut the skin by pulling out.
 - the femoral head should be removed from the acetabulum by cutting the ligament of the head of femur.
- c) connect the two incisions with an incision along the ventral midline extending from mandibular symphysis to anus.
- d) reflect skin dorsally.
- e) open abdominal cavity by incising through the dorsal abdominal musculature and extending your incision downward following the rib cage.
- f) puncture a hole through the diaphragm and listen for air to enter the thoracic cavity (if no air enters - pneumothorax).
- g) make an incision in diaphragm from the sternum dorsally.
- h) remove the ribs with pruning shears, or a smaller instrument, depending on animal size.
- i) examine viscera. At this time sample organs for microbes (eg bacteria, viruses, parasites)

3. REMOVAL AND EXAMINATION OF THE THORACIC VISCERA:

- a) separate mandibular symphysis.
- b) reflect the tongue distally.
- c) section through the hyoid bones to reflect the tongue, oesophagus, and trachea with larynx. Sectioning through the hyoid bones can be difficult. In larger or older animals the hyoid bones are often calcified. It is necessary to slide your knife into the "V" formed by the cartilages of the hyoid bones and the projections of the thyroid cartilages, approaching this junction from the caudal side. The knife should slide into the softer cartilaginous junctions and "cut with ease". This is the area where most cuts occur to the student and/or pathologist - so be CAREFUL!!!
- d) remove both thyroids and parathyroids. Examine and place in formalin.
- e) reflect trachea, oesophagus, and tongue from the soft tissues of the neck.
- f) remove the lungs, thymus, and heart from the thoracic cavity.
 - loosen the lungs and pericardium from their attachments by making three incisions:
 - 1 - along ventral margin of spinal column
 - 2 - along dorsal margin of sternum (try not to incise pericardial sac)
 - 3 - along cranial surface of diaphragm incising oesophagus and vena cava
- g) open esophagus with either the tip of a knife or a pair of scissors, beginning at cranial end and continuing to its caudal termination. Examine for lesions & take sample for histopath.
- h) open larynx / trachea along dorsal cartilaginous junctions to level of the tracheal bifurcation and continue into main bronchi of several lobes. Lesions to look for: pneumonia, edema, congestion, parasites, tumours, thrombi within vessels. Sections for histopathology.
- i) examine the bronchial and mediastinal lymph nodes. Sections for histopathology.
- j) examination of the heart, pericardial sac and major blood vessels: Examine the pericardial fluid if you haven't already done so. Position the heart with the left ventricle on the right and the right ventricle on the left with the left longitudinal coronary groove running vertically at an angle from your left to your right. You will open the heart in the same manner as the blood flows. The first incision is therefore transversely through the right atrium. Remove blood clots and look down to the right atrioventricular valves. Notice if the valves are competent or contain proliferations. Next cutting along the septa, open the right ventricle and extend your incision through the pulmonic valve into pulmonary artery. Examine endocardium and myocardium at this time. Section through left atrium and examine left AV valve for lesions. Section through centre of AV valves to heart apex. Then, following the septum, extend your incision through the aortic valve. The heart can then be washed with water. Samples of the heart for histopathology should include a section of left ventricle, right ventricle with AV valve and a section of the septum. A detailed protocol for measuring specific myocardial measurements has been designed. This protocol may be used if significant pathological changes suggest its necessity. Sectioning the heart in the longitudinal echo-plane may also be done.

4. EXAMINATION OF THE ABDOMINAL VISCERA:

- a) observe the abdominal organs for displacement or torsion.
- b) remove spleen, examine, and sample for histopathology.
- c) test the patency of the bile duct by putting pressure on the gall bladder and checking for movement of bile.
- d) remove the stomach, small intestines, caecum, large intestine, and mesenteric lymph nodes. In small animals, horses, pigs, and non-autolysed ruminants the intestines should be cut and removed at the mesenteric attachment. In autolysed ruminants the entire mesentery and intestines may be removed without stripping the intestines. Open the stomach and examine the contents. Examine the mucosal surface for areas of ulceration and congestion. Sample for histopathology. Take samples of small intestine for bacterial and/or viral cultures if necessary. A sample from large intestine may be submitted for parasitological examination. Depending on size and state of autolysis, the entire small intestine may not necessarily be opened, but please spot check areas and sample duodenum, jejunum, and ileum for histopathology. Examine caecum and large intestine and sample for histopathology. Examine mesenteric lymph nodes, sample for histopathology.
- e) remove, section, examine and sample adrenal glands.
- f) remove liver and gall bladder and examine the capsular surface. Make several incisions through liver. Open gall bladder. Take sections of liver and gall bladder for histopathology.
- g) remove kidneys, section, and examine. The sample for histopathology should include cortex and medulla. Attempt to remove the capsule from the underlying cortex.
- h) examine the urinary bladder. You may want to save urine for bacterial culture or urinalysis.
- i) examine the genital tract. Examine and sample sections of uterus and ovaries. Examine and sample sections of testicle and prostate. Examine and sample mammary glands.

5. EXAMINATION OF JOINTS AND MUSCULATURE:

- a) open and examine as many joints as are necessary.
- b) examine several large muscle masses.

6. EXAMINATION OF THE BRAIN AND SPINAL CORD:

- a) brain and spinal cord removal will be demonstrated. There are several acceptable methods available for removal of brain and spinal cord.
- b) the pituitary and trigeminal ganglia should also be examined and placed in formalin.

COMMENT: This is only a brief description of basic necropsy techniques. As you will see there are many modifications made in this basic procedure as specific organs are affected with disease. Each pathologist has developed their own technique which will vary from the above description.

TISSUE PRESERVATION FOR HISTOLOGIC EVALUATION

1. 10% buffered neutral formalin (10% BNF)

Probably the best routine fixative, though penetrates tissue slowly (~5 mm/24 hours).

Commercial Formaldehyde (37-40%)	100 mls
Distilled water	900 mls
sodium phosphate monobasic	4.0 g
sodium phosphate dibasic (anhydrous)	6.5 g (pH should be 7.2 ± 0.5)

Fix tissue slices 24 - 48 hours at room temperature.

2. Bouins fluid - picric acid base

USES: Endocrine tissues (especially pancreas and pituitary)
Viral Diseases - Demonstrate viral inclusion bodies
Eyes, uterine biopsies - rapid fixation

DISADVANTAGES: Tissues become brittle after 24 hours (tissues need to be washed to remove picric acid and then placed in 50% ethanol).

3. Alcohol

Causes severe dehydration and is not a good fixative.

4. Fixatives for Electron Microscopy

Various fixatives are available for EM processing; most include glutaraldehyde or paraformaldehyde. These fixatives are expensive and may contain carcinogens. Contact the laboratory performing EM for the appropriate fixative.

5. Miscellaneous Fixatives

Some laboratories prefer that your clinic use their fixative. Contact your diagnostic service to see if fixatives are provided.

Notes:

- regardless of particular fixative, consider tissue thickness and total fixative volume: 6 to 10 mm maximum thickness (except eye, brain, spinal cord - fix whole)
- fixative volume 10 times tissue volume.
- handle tissue carefully prior to fixation; do not stretch, squeeze, cut with dull instruments or rinse excessively with tap water.
- freezing - the size of ice crystals formed in tissue is proportional to the length of time necessary to freeze; ie, snap frozen specimens (liquid nitrogen) have few freezing artifacts and are useful for histochemical staining, but carcasses that freeze outdoors or in home freezers result in moderate freezing artifacts ("making interpretation difficult").