FACT SHEET 7

FINNPathologists

The Veterinary Pathologists

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Performing post mortem examinations in small animal practice

This fact sheet aims to provide a guide to performing post mortem examinations (PME) in small animal practice, together with lists of equipment required and how to collect the best quality samples for further tests such as histopathology, microbiology and toxicology. Performing a thorough PME in practice may seem a little daunting at first, but with adequate time, a systematic approach and the right equipment it is entirely practical to do. Remember that freeze-thaw artefact will severely affect the quality of histopathology samples and should be avoided wherever possible.

Before you start….

1. You must obtain fully informed consent from the owner, with a signed consent form (owners should also be aware of the fact they cannot take the animal's remains home and alternative arrangements should be made).

2. You should have a full clinical history, including signalment, previous conditions, clinical signs, current treatments and details about the time and nature of the death (via euthanasia, unwitnessed or accompanied by any signs such as seizures).

3. Remember to consider the health and safety implications – to yourself, other staff and animals. Personal protective equipment (PPE) including disposable gloves, aprons and ideally cut-proof gloves should be available. Potentially zoonotic or highly infectious diseases should only be dealt with somewhere which has the appropriate containment facilities.

4. Is it a case with possible legal implications? If so, it may also be most appropriate for it to be dealt with by a professional veterinary pathologist (e.g. forensic cases, potential welfare cases).

5. Do you have the correct equipment to hand (see table), adequate time and a suitable area in which to perform the PME, i.e. on a raised table in a well-lit room and where thorough cleaning and disinfection are possible. Assistance from a veterinary nurse or EMS student is also very helpful for note-taking and photography.

Note-taking is important; remember to describe any gross findings and record them on the submission forms. Laboratories are also happy to receive digital photographs; remember to include an indication of scale, such as a ruler. Consider recording weights of organs, such as the thymus and heart.

Equipment:

- Post mortem knife
- Scissors (sharp and blunt ended)
- Rat tooth forceps
- Scalpel blade plus holder
- Sample pots, empty and with formalin
- Microbial swabs
- Camera
- Ruler
- Note-taking equipment
- Personal protective equipment
- Cutting board
- Bone cutters, T-piece, saw
- Scales
- Syringes, measuring cylinder
- Glass slides, coverslips, tape and appropriate stains for cytology samples and wet preparations

Part 1 - External examination

- Weigh the animal
- Remove and record any collars, bandages or other materials
- Check for any microchips
- Note the body condition score
- Record any presence of rigor ("stiffness") and/or algor mortis ("coldness")
- Examine the external structures and surfaces including:the coat (clip patches, alopecia, venepuncture sites)
    - skin (petechiae, bruises, icterus, oedema, lesions, tattoos, cutaneous tumours, scars)
    - mucous membranes (pallor, icterus, petechiae)
    - eyes (including any discharge)
    - orifices (nares, ears, mouth including teeth, tongue and pharynx, anus, urogenital – discharges, faecal staining)
- Note any bony malformations, fractures or palpable masses (consider radiography)
- Examine the feet and the nails for evidence of scuffing

Part 2 - Opening the body cavities

- Place the animal on its back
- Make incisions on both sides between the thoracic wall and the forelimbs (figure 1, cut 1)
- Reflect the forelimbs away from the body wall, laying them flat to stabilise the body in dorsal recumbency (figure 2, cut 1)
- Reflect the pelvic limbs in a similar way, by dissecting open the hip joints and laying the pelvic limbs out flat (figures 1 and 2, cut 1)
- Make a ventral midline incision through the skin from the mandibular symphysis to the pubis (figure 2, cut 2)
- Reflect the skin back from this midline incision by cutting through the subcutaneous fat (figure 2, cut 3); check the subcutaneous adipose tissue for any evidence of oedema or bruising
- Slice through, examine and collect any superficial lymph nodes required (peripheral lymph nodes can be difficult to locate unless they are enlarged)
- Make a full incision through the ventral midline from the xiphoid process to the pubis - the linea alba should be elevated from the abdominal contents when making the midline incision to avoid inadvertent puncture of the abdominal viscera
- Extend the incision through the left and right sides of the abdominal wall caudal to the diaphragm and costal arch (figure 3 and 4). Be careful not to puncture the thoracic cavity
- Examine the abdominal viscera in situ and check they are:
    - intact
    - the right size
    - in the right location
- Make a note of any abnormal fluid present in the abdomen:
    - approximate volume, colour, consistency and clarity
    - take a sample
- Make a small stab incision through the diaphragm to check for negative pressure in the thorax - observe and listen for the influx of air – you should see the diaphragm move towards the abdominal cavity and become less tense
- Cut a line through the soft tissues down to the bone along both sides of the ribcage. Using bone-cutters, cut through each rib along one side (figure 4) – you should now be able to reflect the sternum and the attached portions of ribs over to reveal the thoracic viscera in situ
- Use a knife to cut through the opposite site of the thoracic wall at the level of the costochondral junction
- Check for any fluid present within the thorax (take a sample if indicated) and for any displacement of organs

Part 3 - Thorax

- Incise between the mandibles through to the oral cavity and pull the tongue through the floor of the mouth (figure 4)
- While applying gentle traction on the tongue, continue to free the tongue, oropharynx and larynx from their surrounding connective tissues
- Incise across the soft palate, through the joints in the hyoid apparatus and continue to dissect the connective tissues to remove the oesophagus, trachea, lungs and heart out as one (called the "pluck")
- Cut through the oesophagus, caudal vena cava and aorta at the level of the diaphragm to free the pluck from the thoracic cavity - once the pluck is removed, place it on a separate board or table
- Check the tonsils, lymph nodes and salivary glands
- Identify and remove the thyroid and parathyroid glands (also check for the thymus if it is a young animal)
- Open the oesophagus along the entire length, examine the internal surface and note any contents
- Open the trachea from the larynx to the primary bronchi; again note the internal surface and any contents
- Palpate all the lung lobes and note any external lesions, areas of consolidation or haemorrhage and their pattern of distribution (beware of post mortem change)
- Check the bronchial lymph nodes
- Open along all the major bronchi into the lung parenchyma, again noting any contents (take a swab for microbial culture if respiratory disease is suspected)
- Collect tissue samples for histology from several of the lung lobes, ensuring a good section through the lung including major and minor airways through to the pleural surface
- Note if the pieces of lung float in the formalin (this means they contain air).
- Slice through the remaining lung in parallel cuts at approximately 1 cm intervals (like slicing a loaf of bread) - examine the cut surfaces.

Part 4 - Heart

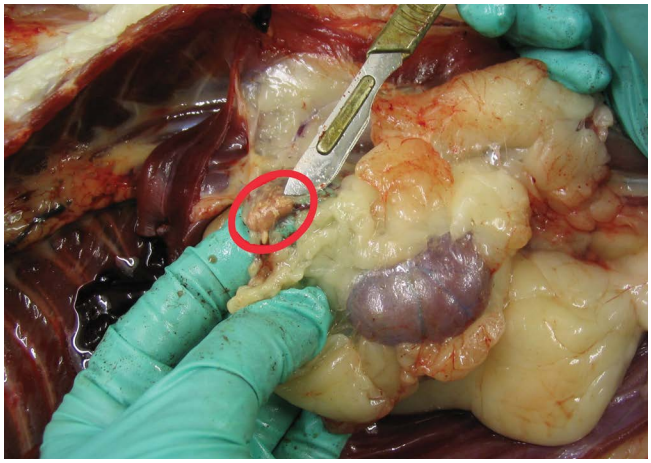
- Incise the pericardial sac, note any abnormalities of the pericardial fluid
- Reflect the sac up over the base of the heart and examine the base of the heart for any external masses or irregularities
- Examine the great vessels and atria, epicardial surface and coronary vessels
- Dissect the heart away from the remaining pluck
- Wash gently in water to remove as much blood as possible from within the cardiac chambers
- Record a weight - the normal weight for most animals is up to 1% of body weight, with exceptions for very young, athletic or cachexic animals
- Identify the right side of the heart; make an incision into the right atrium and then down through the right atrioventricular valve into the right ventricle, just to the edge of the coronary grove
- Open the left side by making an incision directly through the middle of the ventricle starting from the atrium and extending to the apex, cutting through the left atrioventricular valve
- Rinse the endocardial surface thoroughly and examine the endocardium (pallor, depressions, thickening or discolouration)
- Examine the atrioventricular valves (nodules, misshaped or thickening of the valve leaflets)
- Check for any evidence of atrial or ventricular septal defects
- Cut up into the great vessels and examine the aortic and pulmonary valves
- Examine the myocardium on cut surfaces
- Measure the thickness of the right ventricular free wall, interventricular septum and the left ventricular free wall:
  - the normal ratio is approximately 1:3:3 for the RVFW: IVS: LVFW
  - cut through the heart perpendicular to the long axis at approximately one third of the way up from the apex
  - do not take your measurements through the papillary muscles

Minimal samples to collect for histology include a portion of left and right ventricular walls and interventricular septum, including papillary muscles

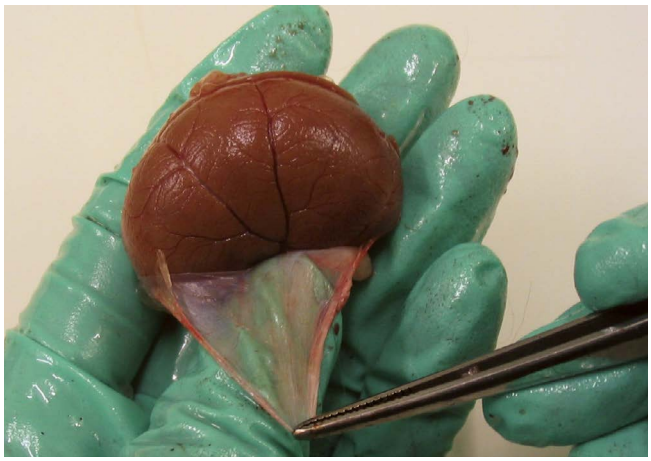
For cases with suspected cardiac disease, or for smaller hearts particularly from cats, you can submit the heart whole once you have obtained the fresh weight.

Part 5 - Abdominal contents

- Locate both adrenal glands (see photo)
- Make a small incision into the duodenum and then check for patency of the bile duct by gently applying pressure to the gall bladder while watching for bile in the duodenum
- Remove the spleen, place it on a separate board or table; serially section (like the loaf of bread again)
- Check the pancreas (both limbs)
- Straighten out the intestines by dissecting through the mesentery, cutting along the mesenteric attachments using scissors. Then cut the stomach free from the oesophagus and also cut through the rectum; remove the gastrointestinal tract from the abdomen
- Cut the liver and gall bladder out of the abdomen and place them on a separate board or table. Open and examine the gall bladder, noting the amount, colour and consistency of the contents. Examine the surface of the liver lobes, note any lesions or masses, colour, size and consistency of the liver (fatty, firm, nodular). Serially section the liver and examine the cut surfaces
- Examine the iliac lymph nodes and abdominal aorta in situ, by opening to the bifurcation
- Free both kidneys from their attachments, bluntly dissect the ureters and urinary bladder and incise through the urethra. Once removed, incise and reflect the renal capsules to the hilus (see photo). Section through both kidneys to the renal pelvis, cutting longitudinally (do not hold the kidneys in your hand when cutting - place them on a board)
- Note the contents of the urinary bladder and open to examine internal surface
- Open the urethra, check the prostate gland if male
- For entire males, section the testes longitudinally
- For entire females, remove the ovaries and uterus and incise to check internal surfaces of the genital tract
- Last of all, open the stomach along the greater curvature, noting the contents. Then remove the contents and examine the internal surface. Open along the small intestines using scissors to the large colon and rectum; again note the contents and examine the internal and serosal surfaces and the mesenteric lymph nodes



Locating the adrenal glands

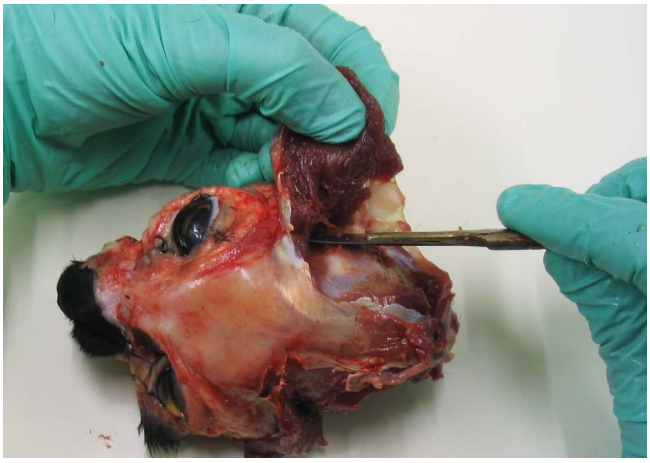


Examining the kidneys: incise and reflect the renal capsules to the hilus, checking for any adhesions between the capsule and the kidney, and for any lesions visible on the surface, for example, nodules, fibrosis, depressions, infarcts.

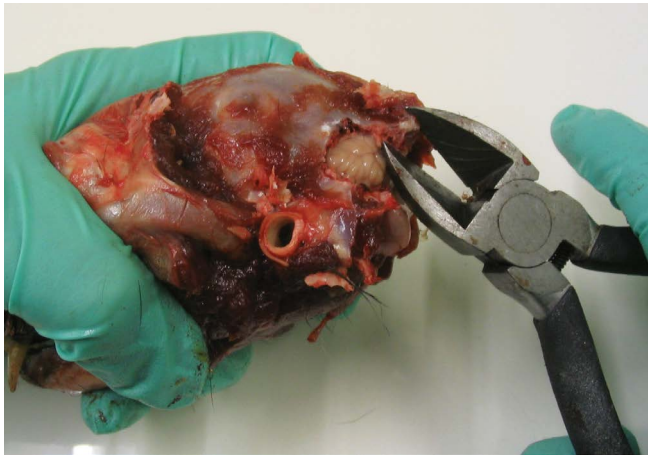
Part 6 – Joints, nervous system, eyes

- Carefully open the hip, stifle, shoulder and elbow joints (and others if indicated)
- Look for:
  - abnormal synovial fluid
  - ruptured, stretched or frayed ligaments
  - erosion and ulceration of articular cartilage
  - thickened joint capsules
  - osteophyte formation
  - proliferative or thickened synovium
- Collect a sample of bone marrow, for example from the mid-shaft of the femur
- Remove the head from the body at the atlanto-occipital joint

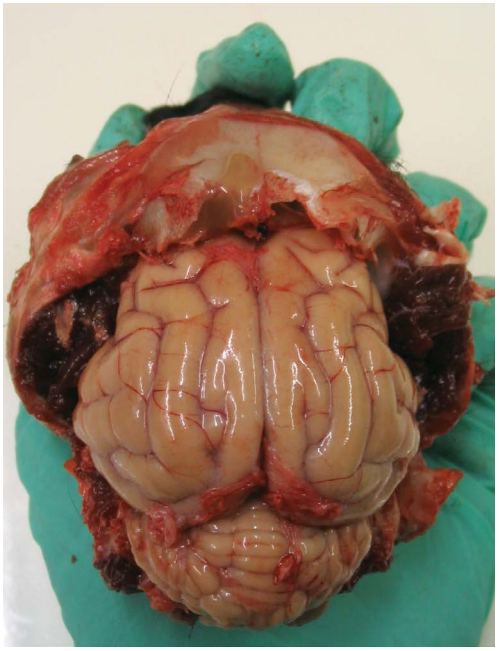
- Make a dorsal midline incision from the nose to the foramen magnum, reflect the skin
- Remove the temporal muscles from the skull to expose the bones (see photo)
- Make cuts through the skull, either with an oscillating saw or a hacksaw (make sure the head is securely held for this, for example in a vice or wrapped in a towel)
  - first cut transversely at the anterior limit of the cranial cavity, slightly posterior to the zygomatic arch
  - connect the end of this cut to the foramen magnum.
  - repeat on the opposite side (figure 5)
- Very carefully prise away the calvarium, examining the internal surface
- For feline cases, you can instead use bone cutters to nibble away the skull, starting at the foramen magnum and working forwards to expose the brain (see photos)
- Cut through and reflect the meninges
- Working from back to front, gently elevate the brain (or even better, turn the skull upside down and use gravity to help)
- Section each of the cranial nerves and the pituitary stalk to free the brain
- Transect the olfactory lobes as far cranial as possible
- Fix the brain *whole* in formalin
- Free the pituitary gland and collect
- If indicated, section the head longitudinally to allow full examination of the nasal cavity and frontal sinuses
- To remove the eyes, close the eyelids and use a scalpel to incise through the eyelids around the orbit. Then, with curved scissors, dissect the eyes from the orbits by cutting the extraoccular muscles, connective tissue attachments and the optic nerve. Collect lacrimal gland tissue if indicated and check for any retrobulbar lesions. Fix the eyes whole without incising (formalin is acceptable)



Removing the brain: dorsal midline incision from nose to the foramen magnum, reflecting the skin ventrally, emoving the temporal muscles from the skull to expose the bones of the skull.

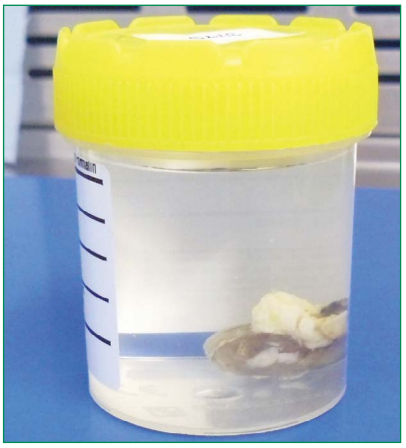


For smaller animals including cats, you can use bone cutters to nibble away the skull, starting at the foramen magnum and working forwards to expose the brain.



Collecting samples for further tests

The samples collected will obviously vary and will be dependent to some extent on the clinical history and gross findings of any particular case. Tissue samples should ideally be collected from all organs and fixed in 10% neutral buffered formalin (you could always store those not submitted in the first instance in case they are required later). A minimal set of samples for histopathology is given in the table, together with samples which can be taken and stored at -20°C where toxicological or PCR testing may be indicated, but not viral isolation. Samples for microbial culture can be stored (fresh not fixed) at 4°C. Consider also taking impression smears of lesions for cytology, urine samples and wet preparations of faeces for microscopy. If submitting cytology samples for examination by a clinical pathologist, do not place slides in same packaging as formalin-fixed samples – the formalin fumes will cause severe artefacts. If viral isolation is indicated it is best to either submit chilled tissues direct to the laboratory or to take samples into virus transport medium and submit. PCR testing for specific viruses will be possible using tissue samples frozen at -20°C. Also remember that Mycobacteria and some conventional bacteria will survive freezing, but most will not.



Appropriate sample pot with label and correct volume of formalin to tissue (10:1)

To achieve the best possible results from histopathology:

- avoid delayed fixation - samples such as the GI tract, pancreas, kidney and brain in particular are prone to rapid autolysis
- use a 10:1 volume ratio of formalin: tissue
- remove body fluids, gut contents etc. prior to fixing but do not scrape the tissue surfaces as you will also remove the surface of the tissues! Gentle washing in water is preferable
- take samples no more than 1cm in thickness - formalin penetration occurs at approximately 1mm per hour and central areas of large or thicker samples will not fix quickly enough to preserve tissue detail
- if focal lesions are present take sections from both affected and adjacent normal tissue
- handle the tissues as gently as possible and do not squeeze or crush them with forceps
- always remember to label all your sample pots!
- avoid using sample pots with narrow necks or lids; samples can become entrapped in such pots as tissues increase in size during fixation
- when posting samples remember the packaging must comply with the postal regulations for pathological materials

Figure legends

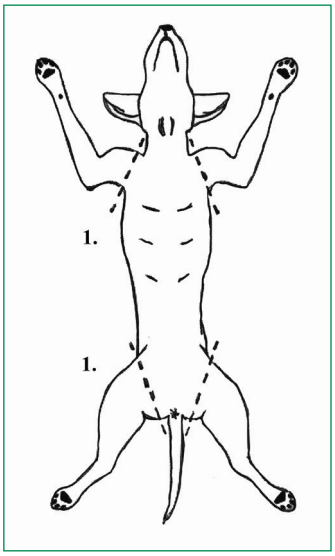


Figure 1.

Place the animal on its back and stabilise the body by making incisions in both axillae and both hip joints (**cut 1**).

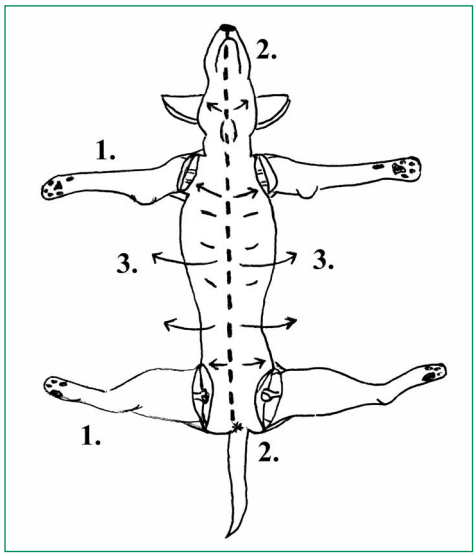


Figure 2.

Reflect the forelimbs and the hind limbs away from the body wall and lay them flat to stabilise the body in a steady position (**cut 1**). Make a ventral midline incision through the skin from the mandibular symphysis to the pubis (**cut 2**). Reflect the skin back from the incision by cutting through the subcutaneous fat (**cut 3, arrows**).

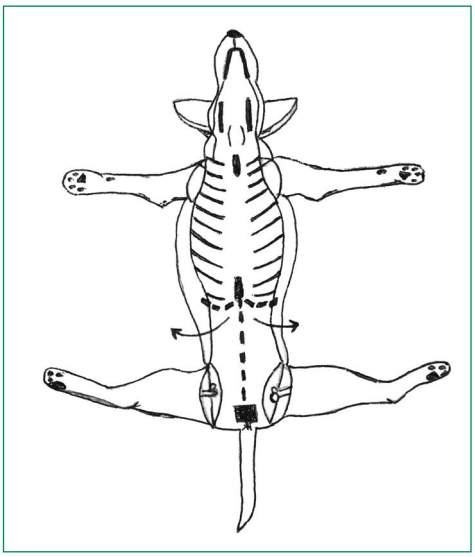


Figure 3.

Make a full incision through the ventral midline from the xiphoid process to the pubis into the abdominal cavity. Extend the incision along the posterior margin of the last ribs on both sides and fold out the abdominal wall to expose the abdominal viscera in situ (**arrows**).

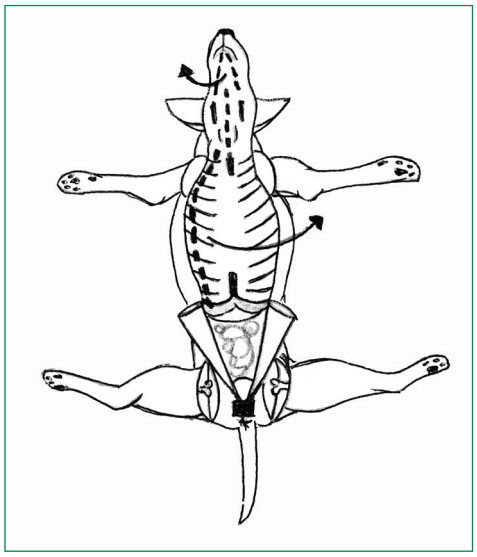
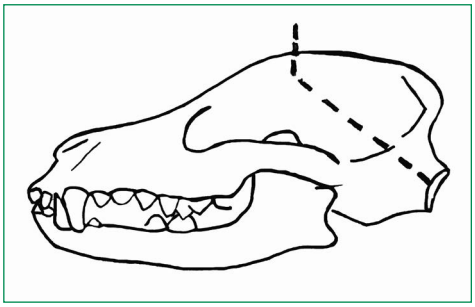


Figure 4.

Cut a line through the soft tissues down to the bone and then using bone-cutters, cut through each rib along one side. Reflect the sternum and the attached portions of ribs over to reveal the thoracic viscera in situ (**arrow**). Incise through the soft tissues between the mandibles through to the oral cavity and pull the tongue through the floor of the mouth (arrow).

Figure 5.

To expose the brain, make cuts through the skull, either with an oscillating saw or a hacksaw; first cut transversely at the anterior limit of the cranial cavity, slightly posterior to the zygomatic arch. Connect the end of this cut to the foramen magnum on both sides of the skull.



Samples:

Toxicology	Minimal (initial) tissue set for histopathology submission	Cytology and parasitology	Microbial culture	Viral isolation
(fresh, frozen)	(formalin-fixed)		(fresh, refrigerated)	
Liver	Liver	Direct impression smears of lesions	As indicated, e.g.	As indicated e.g.
Kidney	Heart		Fluids	1cm^3 of:
Lung	(whole or 3 samples - RVFW, IVS, LVFW)		Tissues	Lung
Fat	Lung (more than 1 lobe ideally)		Swabs	Liver
Stomach contents	Kidney	Faecal samples for parasitology		Kidney
Urine if present	Spleen			Intestine
	GI plus pancreas			Brain
	(duodenum, jejunum, ileum, colon) Any gross lesions (indicate anatomical location)			

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