

Detailed/Organ Specific Sampling Guidelines

Tissue Submissions

DO NOT FREEZE SPECIMENS FOR HISTOPATHOLOGY

Freezing produces artifacts in tissues, which generally result in the sections being unsuitable for interpretation. Do not place formalinized samples in direct contact with ice bags OR with other frozen specimens as this may result in the freezing of the fixed tissue.

Fixation

10% phosphate buffered formalin fixation of choice

RECIPE:

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

- 1) Place in fixative as soon as possible.
- 2) Fix tissue slices within 24 – 48 hours.
Tissue volume should be less than 1/10th that of the formalin solution.
- 3) Use wide-mouthed, leak-proof containers.
Do not use narrow necked or glass jars: A tissue which is fresh and pliable can easily be put into a narrow-mouthed jar, but it becomes hard when fixed and cannot be easily removed.
- 4) Alternatively, tissues can be fixed in formalin solution for 24 – 48 hours, removed from solution, wrapped in formalin-soaked gauze sponge, placed in a plastic bag, and sealed for shipment. This technique decreases the probability of spillage and leakage of formalin during mailing.
- 5) Special Histopathology Requests: Some samples require different fixation or handling procedures. Examples include eye and muscle biopsy samples. Repeated samples for electron microscopic examination requested by the pathologist may necessitate different fixation. Please contact the laboratory prior to taking these samples.

Describe Gross Pathological Findings and Clinical History

- a) Record lesions, include extent, colour, consistency
- b) Report negative findings if relevant. An example could be the absence of gross brain lesions in an animal which had neurological signs.

Tumour Submissions

- a) Indicate whether the tumour appears clinically malignant or benign.
- b) Bone lesions – submit pertinent radiographs (will be returned) and/or detailed radiographic description.

Skin Biopsies

- a) Require careful selection and handling.
- b) Do not shave biopsy sites, as the hairs are useful guidelines for proper plane of section.
- c) Select recent active lesions and margins of lesions incorporating normal skin. If a bullous skin disease (pemphigus) is suspected the active lesion (such as an actual blister) is required for definitive histological diagnosis.
- d) Complete significant information (breed, sex, distribution of lesions, etc.) is vital in dermatology cases.
- e) Flattening the biopsy on a piece of cardboard and floating it upside down in formalin, will result in a fixed tissue that can be properly orientated by the pathologist for sectioning.
- f) Immunohistochemistry staining can be done on tissue blocks either in-house or sent to a referral laboratory. There is an additional fee for this testing.

Hollow Organs

- a) Examples: intestine, urinary bladder, uterus
- b) Cut open longitudinally (with care) to ensure fixation of mucosa
- c) Sections of intestine should be fixed within a few minutes after removal.

Solid Viscera

- a) Examples: liver, spleen, kidney
- b) Cut in slices perpendicular to the surface to demonstrate their anatomic structure. Whenever possible, one surface should consist of the natural boundary of the organ.
- c) Slice structures less than 1 cm in thickness to allow proper fixation.
- d) Submit adequate samples; when possible avoid small minute samples.

Lesion Sampling

- a) If a focal, small lesion, include adjacent normal tissue, as well as the lesion. This will allow identification of the tissue, as well as defining the nature of spread of the lesions.
- b) If a large mass has been biopsied, submit the entire mass with transverse cuts to allow formalin penetration. If this is not feasible, submit appropriate sized sections from several different areas.

Eye submissions

- a) Appropriate fixation solutions for the globe include 10% buffered formaldehyde or Bouin's fluid.
- b) Please leave enucleated globes intact (incision causes collapse and distortion, hampering gross evaluation)

- c) To improve intraocular penetration, globes fixed in formaldehyde should receive an intravitreal formaldehyde injection (0.5-1.0 ml) with the needle inserted through the sclera near the optic nerve.
- d) If extensive orbital tissues are removed with the globe, these tissues should be trimmed (unless involved in the disease process) to allow proper fixation of the globe.

Samples from a Necropsy

- a) Submit sections of abnormal tissue and organs.
- b) Submit samples of major organs: lung, heart, liver, kidney.

Brain Submission

Brains submitted for pathological examination may be submitted intact and immersed in 10 times volume of 10% formalin. Better fixation, and therefore examination, can be obtained by prefixing the entire brain for two days in a large container in 10% formalin and then shipping the entire brain in a smaller container with a small volume of 10% formalin. This method is preferred.

Heart Submission

The heart may be submitted in its entirety, particularly if a malformation is suspected. Rinse the chambers with water and fill with 10% formalin.

Other Helpful Information

It is always wise to freeze and hold portions of organs, so that further tests such as virology, bacteriology or toxicology may be carried out if necessary. When submitting formalized tissues indicate whether frozen portions are being held.