

Why take tissue samples for histopathology?

- Taking tissue samples for histopathology is an inexpensive and quick way to deliver additional important information related to disease identification.
- Histopathology (the collection and microscopic examination of tissue in order to help identify a disease) should be part of any disease investigation, particularly when necropsy (post mortem) fails to identify the cause of death or further confirmation is required.



Procedure

Factors influencing sample quality

- If incorrect sampling procedures are used, it can delay the identification of a disease.
- Tissue must originate from fresh dead birds and must not be frozen, as this will compromise sample quality.
- Collect tissue samples that contain both healthy and diseased areas.
- Tissue samples need to be immersed in a special preservative solution (buffered formalin) to “fix” them (preserve them as close to their natural state as possible). This prevents post mortem decay (autolysis and putrefication).
- The size of the tissue sample influences how quickly it is fixed.

Equipment Needed for Sampling

- Necropsy gloves
- Sharp scissors
- Scalpel
- Forceps
- Leakproof jar with screw cap
- 10% buffered formalin
- Ruler (for measuring sample size) (optional)
- Small petri dish (optional)

Sampling equipment



Example of a leakproof jar with screw cap and 10% Buffered Formalin



Note - If commercial buffered formalin is not available, it can be made using the following method: (Ingredients and quantities are identified in the table)

Ingredients for 10% Buffered Formalin.

Chemical	Quantity
Formalin (37%)	100 ml (3.38 oz)
Distilled water (or tap water)	900 ml (30.43 oz)
Sodium dihydrogen phosphate, monohydrate (H_2NaO_4P)	4.0g (0.009 lb)
Disodium hydrogen phosphate, anhydrous (Na_2HPO_4)	6.5g (0.01 lb)

Place into a chemical safe container with the label "Caution Poisonous – 10% Buffered Formalin".

- If these components are not available, one part of 37% formaldehyde mixed with 9 parts water can be used as a solution, but please note that this may compromise the quality of tissue fixation and the outcome of the results.

Things to Remember Before Sampling

- Thickness of the tissue should not exceed 5mm (0.2 inches).
- The fixative (Buffered Formalin) solution should be 10 times more than the volume of the tissue sample. For example, if the tissue is 5 x 10 x 20 mm, then it has a volume of 1 cm³ (= 0.06 in³ or 1 cc or 1 ml), and you would need 10 cm³ (= 0.6 in³ or 10 cc or 10 ml) of fixative to fix this tissue.
- The tissue should stay immersed in the fixative for at least 48 hours before shipping to the laboratory.
- Changing the fixative may be necessary. Bloody tissues like spleen, liver or lungs, or large tissue samples may contain considerable amounts of blood at the time of sampling. If the fixative becomes murky, it needs replacing with fresh solution.
- Make sure tissue jars are labelled correctly to avoid confusion.

Histopathology sampling procedure

- Step 1** Using a scalpel and tissue forceps, remove a 5mm (0.2 in) piece of tissue from a fresh dead bird, including both diseased and healthy tissue in the sample.

Example of correct tissue thickness for sampling.



- Step 2** Place the tissue sample in a jar of fixative with the sample information, ensuring that the volume of fixative is 10 times that of the sample.

An example of a tissue sample preserved in 10 times the volume of fixative.



Note - Multiple samples may be placed into the same fixative jar as long as they are properly labelled with each samples description to avoid confusion.

Example of multiple specimens within the same fixative jar.



Shipping samples to the laboratory

- After 48 hrs, pour off most of the fixative from the sample jar, leaving just enough to cover the specimens.
- Re-seal the sample jar with a leak-proof aid such as parafilm (commercially available through a scientific supplier) or wax to ensure that the jar does not leak during shipping.
- Contact the laboratory you are shipping to in advance and make sure all the required paper work is included. If shipping internationally, make sure to comply with all laws and obtain the proper shipping permits.
- Make sure that all submission forms are filled in correctly with the appropriate information (name, farm, country, date, etc.).
- Include a summary of symptoms and post-mortem findings.