PROPER COLLECTION AND HANDLING OF DIAGNOSTIC SAMPLES

PART 4: TISSUE COLLECTION AND SUBMISSION FOR HISTOPATHOLOGY

HISTOPATHOLOGY

Histology refers to the evaluation of cells and tissues using a microscope. As a follow-up to the post-mortem exam, histology can be a valuable tool in assessing flock health. Some poultry diseases can only be diagnosed by histopathology. For example, the clinical presentation of infectious laryngotracheitis virus or wet pox within a flock can be virtually identical, but the diseases cause distinctly different and characteristic histopathologic changes that allow a definitive diagnosis.

Successful use of histopathology as a diagnostic practice requires the availability of appropriately selected and preserved samples.

Figure 1. After the tissue samples have been processed and histological sections placed onto glass slides, a trained avian pathologist examines the tissue sections to look for evidence of disease.

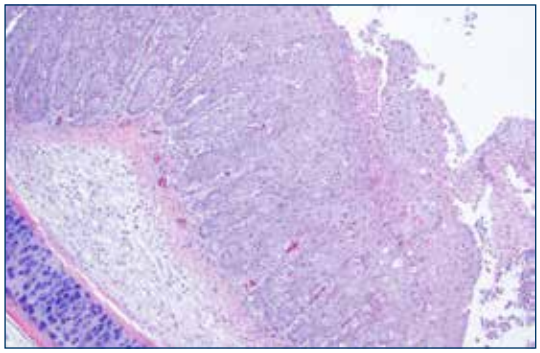

Figure 2. Microscopic view of the tissue of the trachea.

Sample Collection

Collect specimens for histopathology as soon as possible after death to avoid deterioration of tissues. Fresh tissue samples from birds humanely euthanized immediately prior to post-mortem examination provide the best quality slides. If mortality must be used for tissue collection, they should be determined to be fresh as possible, and not decomposed.

Do not collect samples from birds that have been previously frozen. The freeze and thaw processes can disrupt cellular features, leading to poor quality slides.

Samples should be collected using a scalpel or razor blade that is sharp and sterile (Figure 5). Avoid using scissors, as they can crush tissue and destroy microscopic details.

An individual sample should be no larger than 1 cm^3 (1x1x1 cm) to allow for adequate penetration of the tissue with fixative. Larger pieces of tissue will decompose in the center before adequate penetration by the fixative (formalin).

Figure 4. Equipment used for collection of samples for histopathology.

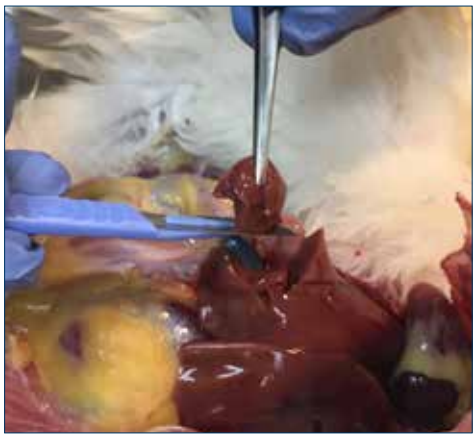

Figure 5. Using a scalpel blade to cut the tissue sample.

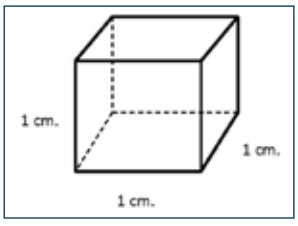

Figure 3. Cubic cm.

Figure 6. For complete and rapid preservation of tissue, the sample should be no larger than 1 cm^3.

Sample Selection

Samples for histopathology should be collected at the time of post-mortem analysis. The selection of samples depends on observations made during the examination. Tumors and other masses, focal discolorations, and organs that are enlarged, atrophied, or otherwise abnormal should be sampled. When a particular disease is suspected based on flock history, tissues associated with that disease may be collected, even if they appear normal (see Table 1). A cross-section of all parts of the affected organ being sampled should be harvested whenever possible.

Tissue cut from the margin of the lesion, collecting both affected and normal tissues, is preferred. Whenever possible, collect healthy-appearing tissue of the same for comparison.

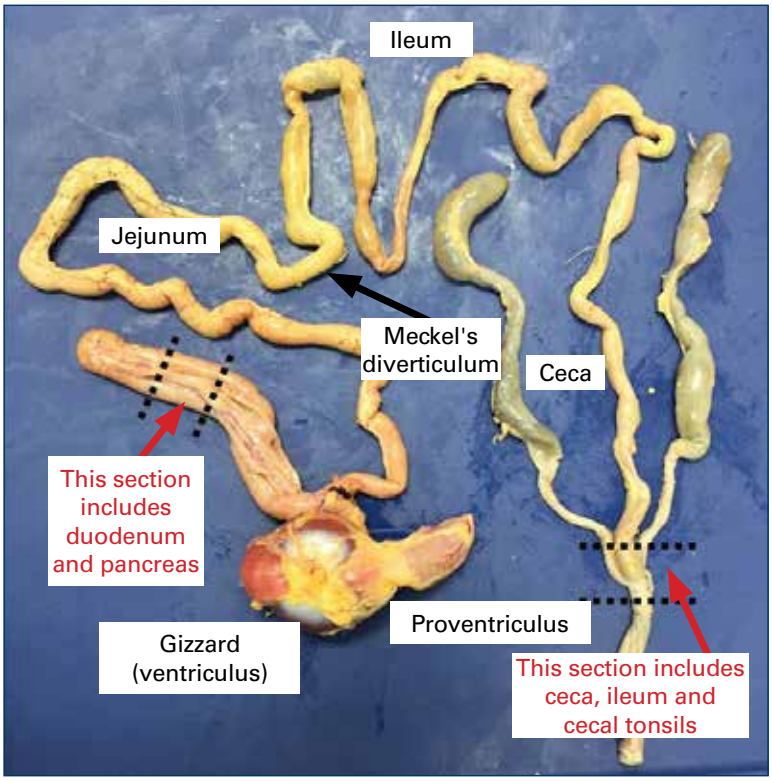

Figure 7. Routine sample sites from the gastrointestinal tract. Cut 2–3 cm sections of intestine in the area of gross lesions or other areas of interest.

Sampling for Specific Diseases

When there is concern for a particular disease based on regional risk, a suspicious result on surveillance testing, or clinical signs in the flock, specific tissues should be collected. Table 1 provides examples of some diseases of concern and the special samples that should be taken.

Disease of Concern	Samples Needed
Gumboro (IBD)	• Bursa of Fabricius, Thymus
Infectious Laryngotracheitis	• Trachea • Larynx • Conjunctiva
Marek's Disease	• Sciatic Nerve • Brain • Eye • Tumors
Wet pox	• Trachea • Larynx
Enteritis (coccidia, focal duodenal necrosis)	• Portions of the gastrointestinal tract affected

Table 1.

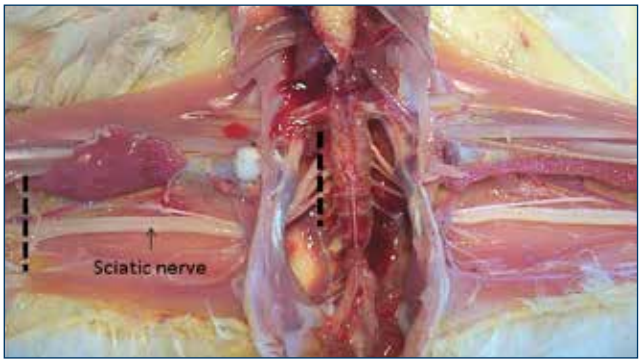

Figure 8. The sciatic nerve of the leg is a sample frequently used for Marek's disease diagnosis. Cut the entire length of the sciatic nerve and place in formalin.

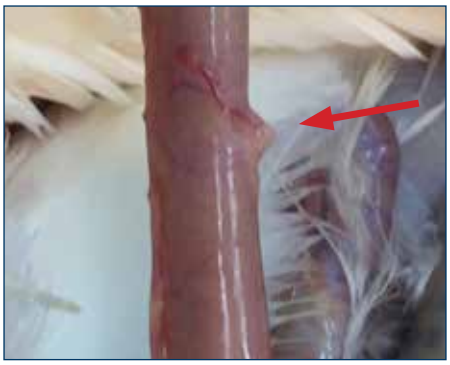

Figure 9. Meckel's diverticulum is the physical landmark dividing the jejunum and ileum.

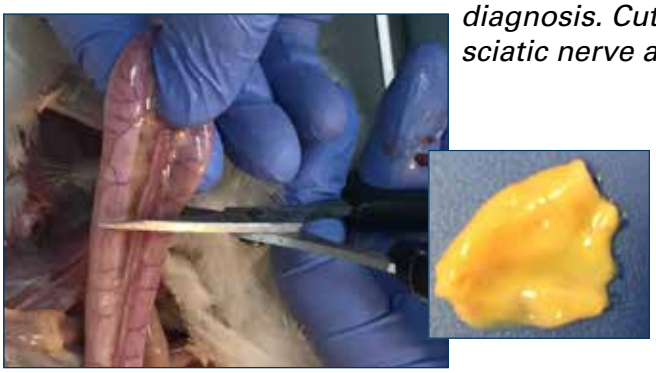

Figure 10. Take a 2–3 cm section of intestine in the area of interest. When collecting intestine sections, gently open lumen of intestine (inset).

Figure 11. Submit the trachea in full; carefully open the trachea along its length.

Figure 12. Carefully remove cranium over the brain with scissors.

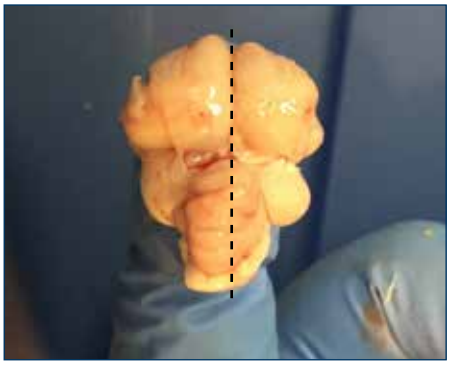

Figure 13. Carefully remove brain and divide longitudinally.

Sample Preservation

Samples should be promptly submerged in a solution of 10% neutral buffered formalin for preservation. The volume of formalin solution in a single container should be at least 10 times the volume of all tissues. Samples must be fully immersed in the solution to be adequately saturated by fixative to prevent deterioration. Lung tissue and other air-containing tissues may be wrapped gently in absorbent cotton to aide immersion. Gently open the lumen of trachea and intestine samples to release trapped air.

After 48 hours in formalin, the tissues are adequately fixed. If necessary for shipping, the formalin can be decanted at this point. Decanted samples should be shipped immediately to minimize the risk of damage to the sample from drying.

Figure 14. Immediately place the tissue into 10% neutral buffered formalin for preservation.

Figure 15. After 48 hours in formalin, tissues are adequately preserved.

If samples may be subject to sub-freezing temperatures during shipping, already fixed samples can be decanted, and re-submerged in an "alcoholic formalin." This will protect against freeze-thaw damage to tissues. For a simple alcoholic formalin mix, combine and pre-mix 6.5 parts pure ethyl alcohol, 2.5 parts distilled water, and 1 part 37% formalin.

Figure 16. After 48 hours, the formalin can be decanted and samples kept in sealable, leak-proof plastic bags.

The formalin-fixed samples can be kept in sealable plastic bags (e.g. Whirl-Pak® bags), or remain in a securely sealed jar with formalin.

If the samples are to be mailed to the laboratory, double-bag the sample to prevent leakage. Remember that formalin is a poison and exposure to the liquid or vapor is harmful to humans.

Figure 17. Whirl-Pak® type bags are sealable, leak-resistant, and can be used for storing and transporting samples.

Sample Submission

When submitting samples to a diagnostic laboratory, it is important to provide thorough and relevant flock information on the laboratory submission form. Critical information that should accompany all diagnostic sample submissions includes:

- Flock identification and location
- Age of flock
- Date of sample collection
- Tissue(s) collected
- Vaccination program
- Flock history, including description of any clinical signs, production problems, and the present level of mortality
- Special shipping regulations may apply for formalin filled containers
- Locally appropriate biohazard labelling on all transport containers
- Appropriate permits for international shipping (e.g. USDA-APHIS permits) if appropriate

This information is vital to the flock veterinarian and diagnostician to make a meaningful interpretation of diagnostic results and provide recommendations to improve flock health and/or production.

Figure 18. Double bagging samples will prevent leakage of the formalin during transportation.

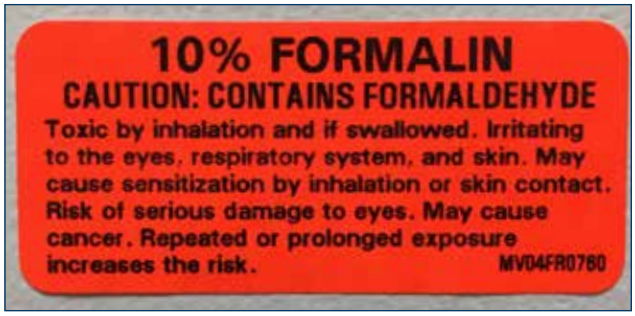

Figure 19. Formalin is harmful to human health. Appropriate biohazard warning labels should be on all containers.

Sample Processing

After arrival at the diagnostic laboratory, the formalin preserved tissues are embedded into a paraffin block, then sectioned with a microtome into thin slices. Tissue slices of this thickness (4 micron) are thin enough to be examined by the pathologist under a light microscope. These slices are fixed on glass slides and stained. Various stains can be used to highlight different cell types, or other aspects of the tissue. The most frequently used stain for disease diagnosis is hematoxylin and eosin (H&E) stain.

REFERENCES

1. Bermudez, Alex J. and Bruce Stewart-Brown. Chapter 1: Principles of Disease Prevention: Diagnosis and Control, "Disease Prevention and Diagnosis". Diseases of Poultry. 13th edition. Ames: Wiley-Blackwell, 2013.
2. USDA-APHIS. United States Veterinary Permit for Importation and Transportation of Controlled Materials and Organisms and Vectors. U.S. Department of Agriculture. 2016.