VETERINARY HISTOPATHOLOGY

MAXIMIZING SAMPLE SUBMISSION FOR OPTIMAL DIAGNOSTIC RESULTS

POULTRY DIAGNOSTIC & RESEARCH CENTER | UNIVERSITY OF GEORGIA | ATHENS, GA

Samples taken for histopathology can provide diagnostic results that are timely and inexpensive for the poultry veterinarians when done properly.

Collect a Representative Tissue Sample

- Select tissue that accurately represents the observed lesion or suspected disease condition.
- That will be Converted from a three-dimensional tissue sample into a thin, stained tissue section that can be evaluated in two-dimensions under a microscope.
- Keep samples small and manageable so they can be properly processed later.

Fix the Tissue Correctly

- Maintain fresh tissue in a state that stabilizes its architecture and chemical components.
- Preserve the sample for histological staining and long-term storage

Best Practices:

- Formaldehyde based fixatives are routinely used in diagnostic setting on the grounds of cost, efficacy, versatility and relative safety.
- Use buffered 10% formaldehyde.
- Ensure the fixative volume is at least 10 times the volume of the tissue
- Place tissue into the fixative, rather than pouring fixative over tissue already in a container

Important Considerations:

- Formaldehyde penetrates tissue at approximately 5 mm per 24 hours
  - Tissue that is too thick will not fix properly in the center
  - Poor fixation can lead to artifacts and reduce diagnostic accuracy
- Allow adequate time for fixation before shipping

Special Situations:

- Encapsulated organs (e.g., spleen, testes) → Cut into the capsule to allow fixative penetration
- Bloody tissues (liver, spleen, lungs) → May require more fixative or changes of fixative
- Large or multiple samples → Changing fixative may improve preservation

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Choose the Right Container

- Use leak-proof containers with wide openings
- Avoid narrow containers—tissues stiffen after fixation and may become stuck
- Improper containers can damage samples during removal or create hazards for laboratory personnel
- Do not send formalin in a non-formalin container

Avoid Using:

- Ziploc-style bags
- Staples (can cause leaks)
- Glass container, Urine cup

Prepare Samples for Shipping

- After adequate fixation, pour off excess fixative
- Place tissues in a sealed, leak-proof container (e.g., Whirl-Pak® bag)
- Prevent drying while complying with shipping regulations:
  - Include a small amount of formalin **OR**
  - Add a formalin-soaked paper towel
- Tissues should remain moist, not submerged, during shipment.

Use Special Fixatives When Needed

- Some tissues require different handling for optimal preservation:
- Eyeballs → Use Davidson's solution
  - Penetrates faster than formaldehyde
  - Prevents retinal detachment artifacts
  - Preserves delicate structures more effectively

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Provide Complete Submission Information

- Accurate interpretation depends heavily on the information provided.
- Include tissue identification, signalment (especially age), clinical history, necropsy findings
- Be specific and included meaningful summaries

Why this matters:

- Some findings are age-related or normal variation
- Context helps determine whether lesions reflect a flock-level issue or an individual outlier

Examples:

Bursal sections are routinely submitted for scoring to the PDRC pathologists. However, samples from birds less than 17 days old have tremendous variation in follicle size and lymphoid content; it is difficult to determine if the changes are significant or normal variation.

Information regarding any gross lesions seen during the necropsy is helpful when submitted fixed tissues. “Tissue for Histo” or "Increase Mortality" is not a reason for submission.

Summaries of necropsy findings (3 out of 4 birds with pneumonia, 3 out of 5 birds with bursal and thymic atrophy, 5 out of 5 birds with multifocal white flat pin point hepatic foci) will help the PDRC pathologist determine if the lesions seen are a flock problem or an individual bird problem that might be an outlier.

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Understand Laboratory Processing

Once samples arrive at the Poultry Diagnostic and Research Center, they undergo a multi-step process.

Trimming

- Samples are reduced to fit into standard cassettes (~40 × 28 mm)

Processing

- Tissue is dehydrated using graded alcohols, cleared with a solvent, infiltrated with wax

Embedding

- Tissue is oriented in a mold and surrounded by wax to form a supportive block.

Sectioning

- Thin sections (~3.5 µm) are cut using a microtome
- Sections are floated on a warm water bath and mounted on slides

Staining

- Routine stain: hematoxylin and eosin (H&E)
- Slides are then coverslipped and prepared for examination

Timing

- Most samples → processed overnight, ready next day
- Bone → may require decalcification, increasing turnaround time
- ILT cases → same-day slides if received by 11:00 AM

Additional Testing Options

- If routine staining is not sufficient, additional diagnostics are available:
  - Special stains (e.g., Giemsa, PAS, GMS, Acid Fast, Trichrome)
  - Immunohistochemistry (CD3, Polyclonal Astrovirus and PAX 5)
  - PCR from formalin-fixed tissues are useful when fresh samples are unavailable (e.g., international submissions)

After Diagnosis

- Slides and paraffin blocks are archived for future use
- Additional sections or tests can be performed later if needed
- Untrimmed tissues are typically kept for at least 30 days
  - Long-term dry storage can reduce sample quality

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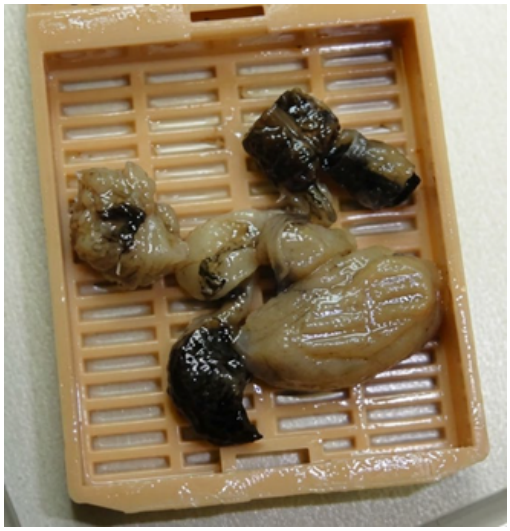
Examples of cases submitted to PDRC when there has been an improper submission of fixed tissues.

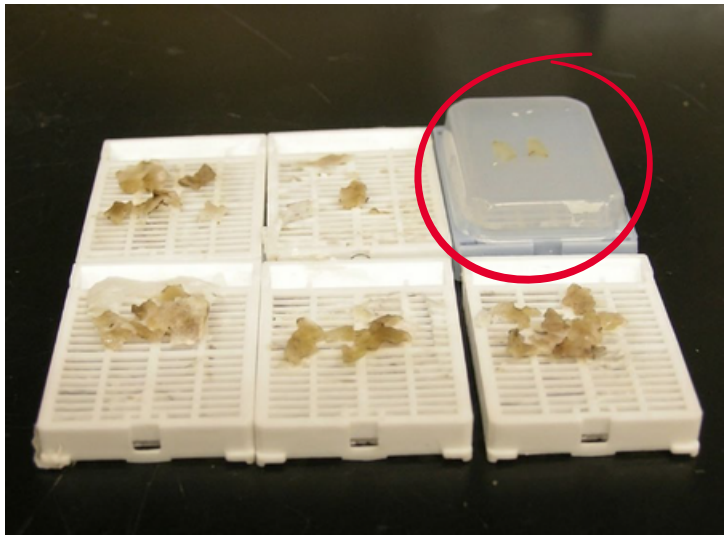
Some samples are submitted already trimmed in cassettes. Tissue too thick for the cassette protrude through the lid and results in impression artifacts as seen in this photo. These impression artifacts can appear in the slide examined by the pathologist.



In some cases, international samples are submitted in wax blocks. Here, the wax melted during transportation, leaving tissues exposed (white cassettes).

Encircled cassette demonstrates the proper amount of wax for comparison. These samples had to be re-embedded before sectioning.

Problems arise if the tissues come off the submitted blocks and more than one case is submitted. Laboratory personnel may not be able to determine which case the tissue belongs to.



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Examples of cases submitted to PDRC when there has been an improper submission of fixed tissues.

These nerves twisted when fixed. The sectioning of samples like this is difficult and may not result in being representative of any microscopic nerve lesions. Place nerves on an index card before submerging them in formalin to keep straight and flat.

These are examples of jars submitted that are overstuff with tissues. The tissues do not have enough formalin to fix properly and will result in artifacts which hinder microscopic examination.

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Remember when submitting histology samples:

1. Proper formalin to tissue ratio (10:1) for fixation.
2. Include any important gross lesions.
3. When submitting neoplasia, include with the mass some normal tissue if possible.
4. When suspecting a viral neoplasia problem, submit a complete set of tissues, not just affected organs. Include brain and eyeballs with optic nerve still attached.

If you are interested in sending samples to the lab or have any additional questions, please contact us at 706-542-5657 or pdrc@uga.edu.

Domestic Submission Form

International Submission Form

International Permit Allantoic Fluid, Serum, and Tissue Samples

Additional resources and submission forms are available on the laboratory website: https://vet.uga.edu/diagnostic-service-labs/pdrc-diagnostic-services

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