

Histopathology Services for Commercial Poultry Production

Veterinary Diagnostic Pathology, LLC

Dr. Frederic J. Hoerr

Fort Valley, Virginia, USA

www.vetdx.com

Dr. Frederic Hoerr



- Education and Training
 - DVM (76), MS (77) and PhD (81)
 - Laboratory of Dr. R. W. Winterfield
 - Board-certified in Veterinary Pathology and Poultry Medicine
 - 38 years experience in histopathology of commercial poultry production
- Current: Veterinary Pathologist, self employed
 - *Veterinary Diagnostic Pathology, LLC* (Founded 2004)
- Experience
 - Diagnostician, Alabama State Diagnostic Laboratories 1980-2012 (Director 1987-2012)
 - Professor Emeritus, Auburn University College of Veterinary Medicine

Poultry Histopathology Services

- General diagnostic problem solving
- System surveys: Immune, Respiratory, Digestive
- Muscle and skeletal pathology
- Tumor histopathology and consultation
- Processing plant quality: skin, muscle, special problems
- Research and Development

Histopathology Reports

- Description of lesions
- Comments regarding lesion patterns and differential diagnoses
- Recommendations for additional testing
 - Viral and bacterial isolation or molecular diagnostics (PCR)
 - Serology
 - Toxin analysis
 - Management and nutrition issues that may impact the lesions that are detected

Veterinary Diagnostic Pathology, LLC

- Facilities
 - Specimen accessioning and gross tissue trimming
 - Slides produced by an accredited pathology laboratory
 - Slide and paraffin block storage
 - Microscope and related equipment
- USDA Import permit is for formalin-fixed tissue and FTA cards only – not fresh tissue
 - FTA cards are accepted and can be forwarded to other testing laboratories in the USA
 - My preference is for the client to send FTA cards directly to testing laboratory (address listing follows)

Available Upon Request

fred.hoerr@gmail.com

- Submission forms
- USDA Permit
- Certificate of Insurance
- Payment by Electronic Funds Transfer information
- Tissue specimen collection guide for disease syndromes
- Contact Dr. Hoerr by email for more information
 - Fees and project quotations
 - Using histopathology to address diagnostic problems
 - Designing surveys for immune, digestive, respiratory problems, etc.
 - Histopathology to support production trials and applied research

Tissue Selection Guidelines

- Refer to Poultry Histopathology Tissue Collection – General Guide (CF.405.1) and 205.1 Poultry Gastrointestinal Sampling for Histopathology
- General Diagnostics
 - Select 3 to 5 live, clinically affected birds for euthanasia and tissue collection
 - 3 to 5 minute maximum delay (minimize autolysis)
 - Very important for intestine
 - Also, select 2-3 recent mortality for tissue collection
 - Select tissues with typical lesions
- At the same time, collect tissue or FTA cards for virology and bacteriology
 - Iced, refrigerated or frozen tissues
 - Proper labeling, packaging and storage of all samples
 - Tissues and samples for toxicology (if needed)
 - Blood for serology

10% Buffered Neutral Formalin Solution (1 Liter)

Formaldehyde 37-40%.....	100.0 ml
Distilled water.....	900.0 ml
Sodium phosphate, monobasic.....	4.0 grams
Sodium phosphate, dibasic (anhydrous)	6.5 grams

Store in properly labeled container. Label as HAZARDOUS chemical. The specimen should be completely submerged in 5-10 times its volume of fixative.

Reference: AFIP Laboratory Methods in Histotechnology, p. 28.

Sources of Prepared Formalin

Avoid Alcohol-Based Formalin Substitutes

- Google Search: “Neutral Buffered Formalin”
- Commercial vendors in the USA
 - Thermo Scientific – good record for not leaking during transport
 - <http://www.thermoscientific.com/ecom/servlet/productsdetail?productId=12933618&groupType=PRODUCT&searchType=0&storeId=11152&from=search>
 - Fisher Scientific
 - <http://www.fishersci.com/ecom/servlet/itemdetail?catalogId=29104&productId=3297312&distype=0&highlightProductsItemsFlag=Y&fromSearch=1&storeId=10652&langId=-1>
 - Leica (Surgipath)
 - <http://www.leica-microsystems.com/biosystems/products/total-histology/consumables/reagents-solutions/fixatives/details/product/10-neutral-buffered-formalin-1/>
 - VWR
 - https://us.vwr.com/store/catalog/product.jsp?catalog_number=16008-002
- Prefilled containers – error on the slide of larger containers.
 - 120ml/60ml fill capacity – small samples
 - 180- 240 ml/120 ml fill capacity – large samples
- Avoid overfilling the container
 - 1:10 ratio of tissue:formalin is optimal
 - 1:5 ratio is acceptable for small pieces of tissue

Tips for Better Histopathology Results

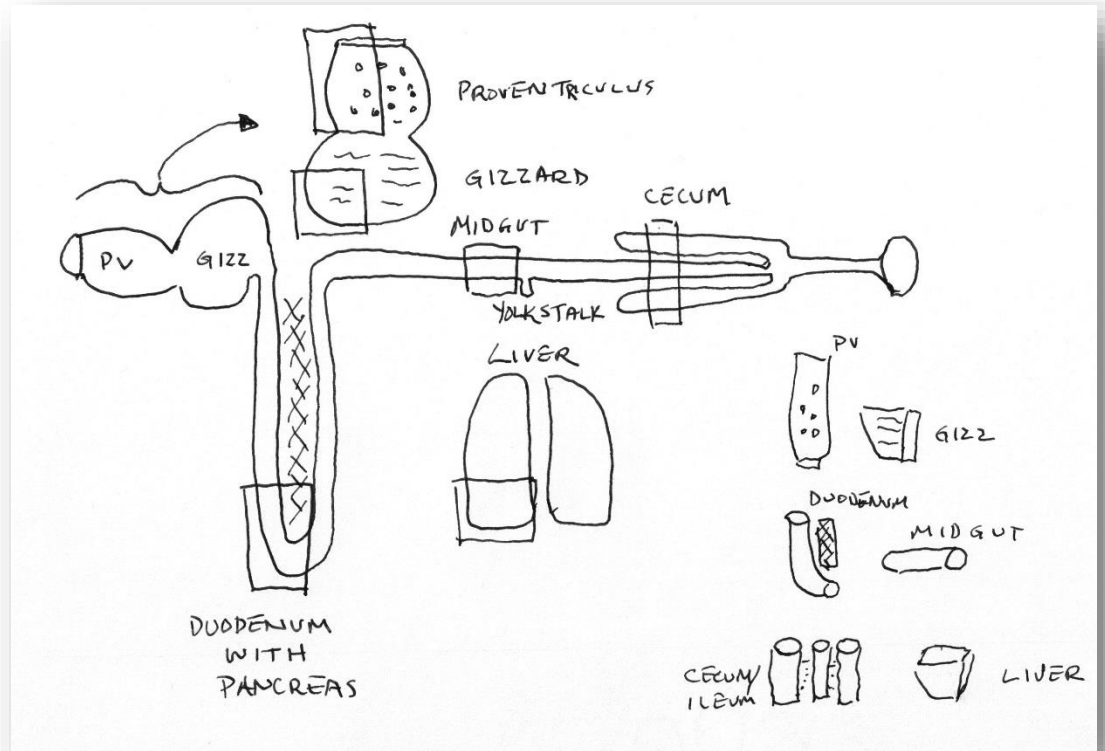
- Use Neutral Buffered Formalin! **Please!**
 - Recommend commercial formalin containers (following page)
 - Non-buffered or concentrated formalin damages villus morphology
- Be gentle with tissue
- Avoid scraping mucosa before putting tissue in formalin
- Avoid freezing formalin tissue
- Avoid allowing the tissue to become dry or hardened
- If applicable, select tissue from clinically affected and recent mortality
- For surveys, collect tissues within 3 to 5 minutes of euthanasia
- Label the container, not the lid; use a *Sharpie*
- Securely close container – *if it leaks before shipping, it will leak during shipping.*
- Provide a list of all flocks submitted; in the desired order of the results & report

Tips and Preferences for Collecting Histopathology Specimens

- Intestine
 - Collect similar anatomic sites from each bird
 - Collect closed (not opened) gut samples about 1-cm in length
 - Keep pancreas with duodenum
 - Midgut: sample near the yolk stalk diverticulum
 - Cecum: sample at midbody; keep jejunum attached
 - Submit cecal tonsils separately
- Respiratory
 - Exudate in the trachea is valuable (LT inclusion bodies)
 - Avoid scraping the tracheal mucosa
 - Include conjunctiva, lung, bursa and thymus, kidney
 - Select obvious, representative lesions for examination

Tissue collection sites

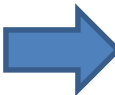
- Collect tissue within 3-5 minutes of euthanasia
- Gut sections 1-1.5 cm in length, CLOSED
- Avoid squeezing tissue (distorts the microanatomy)
- Recommend examining 4 to 5 birds per age group
 - Up to 5 tissues per slide



International Shipment to USA- Checklist

- Fix tissues for a minimum of 3 days before shipping
- Remove 90% formalin; tissue is moist (wet) with formalin.
- (1) Primary sealed container with tissue specimen
- (2) Secondary sealed container with adsorbent material (paper towel).
 - No leaks!
- (3) Tertiary rigid container (cardboard/Styrofoam box) with packing material
- External envelope “**USA Customs Declaration**”
 - Customs declaration form
 - USDA Permit

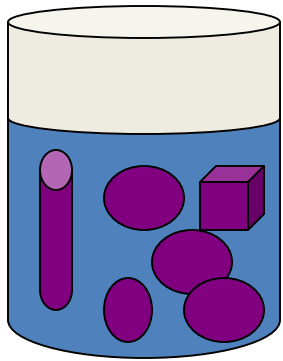
United States Customs Declaration Form – See Attached file

- Place an envelope on the exterior of the package, labeled “**US Customs**”
- **Enclose Two Documents**
 1. USDA Permit
 2. Customs Declaration 

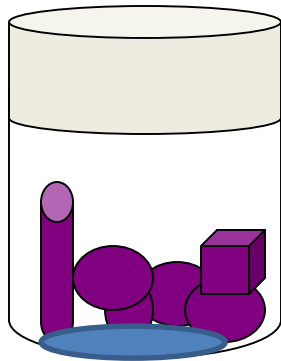
Customs Declaration	
To:	United States Customs Officials
Concerning:	_____ <i>Courier's parcel tracking number</i>
Sent from:	_____ <i>Sender's name on shipping label</i>
This is to confirm that the exported material in this package: 1) Is derived from poultry; 2) Contains no other animal origin material; 3) Did not come in contact with any other animal origin material; and 4) Was fixed in 10% formalin.	
_____ Printed/typed name of responsible person	
_____ Signature of responsible person	_____ Date

1. Primary Container

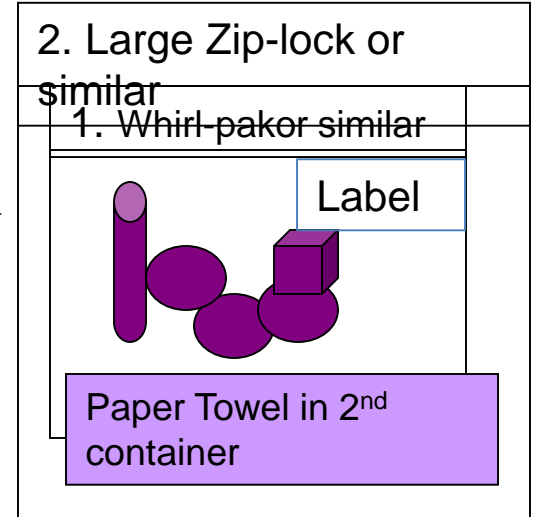
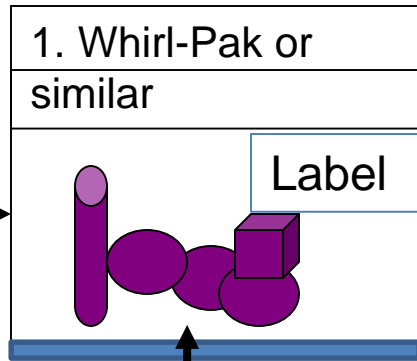
2. Secondary Container



3 days in formalin

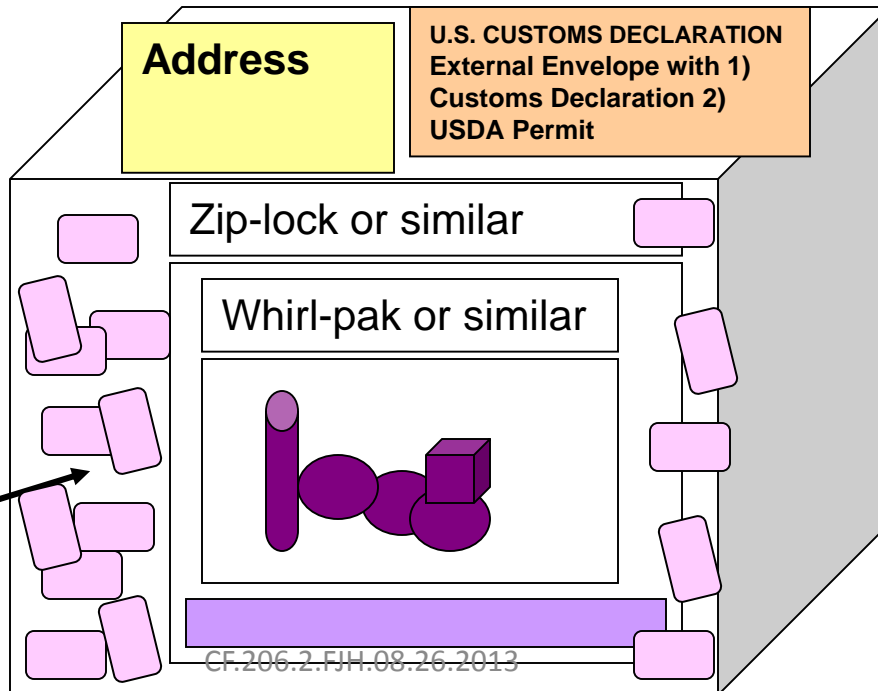


Remove 90% formalin; tissue wet



3. Tertiary Container Rigid material - FedEx, DHL, UPS box

Packing material



Formalin-fixed tissue packing instructions for international shipment to USA.

Primary and Secondary Packaging

(Domestic Sample - Formalin not Removed)



The formalin container is tightly sealed and does not leak. It is surrounded by adsorbent material (paper towel) and placed in a sealed secondary container (Whirl-Pak bag). For international specimens, it is recommended that about 90% of the formalin be removed prior to shipping. **ALTERNATIVELY:** Multiple jars can be placed in one sealed secondary container (with adsorbent), then packaged in a secure box.

Addresses

Formalin-fixed tissues for histopathology

Veterinary Diagnostic Pathology, LLC
638 South Fort Valley Road
Fort Valley, Virginia, 22652 USA

Phone: 540-933-6409

fred.hoerr@gmail.com

www.vetdx.com

FTA cards for Virus, Bacteria, Mycoplasma, and Tumor testing

Send samples directly to:

Poultry Diagnostic and Research Center (PDRC)

953 College Station Road

Athens, Georgia 30605 USA

706.542.5657 Fax: 706.542.0252

E-mail: pdrc@uga.edu

<http://www.avian.uga.edu/diagnostic/dxlab.php>