

VERMONT AGENCY OF AGRICULTURE, FOOD & MARKETS  
FOOD SAFETY CONSUMER PROTECTION DIVISION  
Meat Inspection Service  
MONTPELIER, VT  
Anson Tebbetts, Secretary



# MIS DIRECTIVE

Adopted from FSIS Directive 10230.6 Rev. 1

10230.6 Rev.

1

03/03/2022

## SUBMITTING TISSUE SPECIMENS FOR PATHOLOGIC EVALUATION TO THE LABORATORY

### I. PURPOSE

FSIS is revising this directive to provide Public Health Veterinarians (PHVs) with instructions on how to submit specimens for pathologic evaluation to the FSIS Eastern Laboratory.

### II. CANCELLATION

FSIS/VT Directive 10230.6, *Submitting Tissue Specimens for Pathological or Diagnostic Microbiological Evaluation to the Laboratory*, 1/06/06

### III. SIGNIFICANT CHANGES

A. The Agency is no longer supporting diagnostic microbiological evaluations, except under special circumstances or studies.

B. All pathology submissions are to be made using the Public Health Information System (PHIS), except in cases where there is no access to PHIS.

### IV. BACKGROUND

A. After performing a careful examination and inspection of the carcass and parts, the PHV makes a disposition if abnormal tissues are identified. In most cases, the PHV will make a disposition based on [9 CFR part 311](#), [9 CFR part 381](#), and [9 CFR part 539](#). PHVs may exercise their professional judgment in making the disposition when the regulations do not adequately describe a disease or condition. At their discretion, PHVs may submit specimens for pathologic evaluation to assist in making a disposition if the carcass is retained, or to confirm a disposition if the carcass is already condemned. The PHV combines the organoleptic inspection information with the laboratory results in making a final disposition.

B. PHVs may seek histological diagnostic assistance from the FSIS Eastern Laboratory. Vermont PHVs may also use the New Hampshire Veterinary Diagnostic Laboratory. Histological evaluation may provide a diagnosis, describe the severity or chronicity, or identify etiologic agents. Additionally, histologic findings assist PHVs with interpretation of abnormalities when conducting future postmortem dispositions.

## V. SAMPLING SUPPLIES

A. PHVs are to obtain pathology sampling supplies from the Eastern Laboratory and are to request these supplies via email to [EasternLabPathology@usda.gov](mailto:EasternLabPathology@usda.gov). PHVs are to direct questions regarding how to obtain tools (e.g., knives, cut resistant gloves) not included in the sampling kit to their immediate supervisor.

B. PHVs are to return excess pathology sampling kits or kits containing expired formalin to the Eastern Laboratory for proper disposal. PHVs are not to use the overnight billable label included in the sampling kits to return excess pathology sampling kits or kits containing expired formalin to the lab. PHVs are to instead contact the Eastern Laboratory via email at [SamplingSupplies-EasternLab@usda.gov](mailto:SamplingSupplies-EasternLab@usda.gov) to obtain a return label.

C. PHVs are not to use expired formalin to submit specimens. PHVs are not to pour formalin down the drain.

D. PHVs are to refer to FSIS [Directive 4791.5](#), *Hazard Communication Program* for the hazard communication requirements for chemicals found in sample collection kits.

## VI. SELECTION OF TISSUE SPECIMENS FOR PATHOLOGIC EVALUATION

A. PHVs are to prepare and submit tissue specimens to the laboratory for pathologic evaluation. PHVs are to collect tissue specimens, as necessary, to assist in the disposition determination of a carcass or carcass parts. If PHVs decide to collect tissue specimens, they are to refer to the tissue selection guidance listed in [Table 1](#).

Table 1: Tissue Selection Guidelines

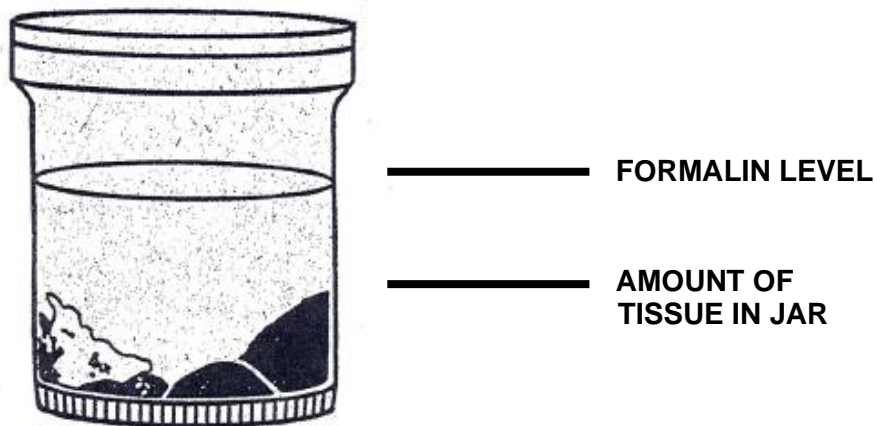
Condition	Representative Samples of Tissues to Submit
<b>Systemic conditions</b>	<ul style="list-style-type: none"><li>• Major visceral organs (liver, kidneys, spleen, heart, lungs)</li><li>• 2-3 lymph nodes draining different areas</li><li>• Grossly identifiable lesions (e.g., abscesses, hemorrhage, necrosis)</li></ul>
<b>Neoplasia</b>	<ul style="list-style-type: none"><li>• Primary mass (include affected tissue and adjacent normal tissue)</li><li>• Lymph nodes that have suspected metastatic lesions</li><li>• Lymph nodes receiving lymphatic drainage from the site of origin of the tumor</li></ul>
<b>Avian leukosis complex</b>	<ul style="list-style-type: none"><li>• Skin</li><li>• Sacrosciatic nerve</li><li>• Organs (liver, spleen, kidney)</li><li>• Bursa of Fabricius</li><li>• Bone marrow</li><li>• Any other tissues with suspicious lesions</li></ul>
<b>Conditions not listed above</b>	<ul style="list-style-type: none"><li>• Tissue specimens from all organs and tissues suspected of having lesions</li><li>• Tissue sections at a point of transition between normal and abnormal tissue</li><li>• Adjacent draining lymph nodes</li></ul>

## VII. PREPARATION OF TISSUE SPECIMENS FOR SUBMISSION

A. PHVs are to preserve tissue specimens for evaluation by placing the tissues in a jar containing formalin kept at room temperature. Additional instructions for sampling and packaging specimens are included on the inside flap of the yellow pathology specimen box. For further assistance, PHVs are to contact the Pathology Branch at the Eastern Laboratory at (706) 546-3556 or via email at [EasternLabPathology@usda.gov](mailto:EasternLabPathology@usda.gov).

B. To ensure that specimens are adequately preserved for pathologic evaluation, PHVs are to:

1. Cut specimens 3/8 inch (9 mm) thick. Thicker tissues will have reduced formalin perfusion. The tissues will not maintain their cellular integrity properly and loss of cellular detail will occur. Incise through the capsule of intact organs such as lymph nodes or the avian spleen before placing in formalin. Avoid exposing specimens to air for a prolonged period of time that would result in drying of the tissue surfaces before immersion in formalin.
2. Place tissues immediately in formalin to preserve cellular detail. Prolonged holding, even at refrigerated temperatures, will result in autolytic changes that may mimic degeneration. Use approximately one part tissue to ten parts formalin. Increasing the tissue to formalin ratio, either by increasing the number of tissues per jar or cutting specimens too large, reduces formalin fixation and results in the loss of cellular detail. If formalin is unavailable, PHVs may refrigerate (do not freeze) tissues to slow autolysis until formalin is available. If additional formalin is needed to adequately preserve tissues from the same carcass, samples can be submitted in jars in more than one pathology box (being sure to clearly identify the jars associated with the same case).



3. Use metal or plastic devices to identify lymph nodes or other affected tissues when differentiation is important (e.g., two different disease processes or metastasis correlation). For example, when submitting two lymph nodes, place one paperclip in one lymph node and two paperclips in the other lymph node for identification purposes. Affected tissue includes the normal surrounding tissue and any lymph nodes draining lymph from the affected area. If the same disease process is suspected in multiple lymph nodes and differentiating these lymph nodes is not essential for the disposition, identifying lymph nodes is not necessary.
4. Store specimens placed in formalin at room temperature. Do **not** place specimens stored in formalin in the freezer or refrigerator. Freezing tissue reduces the microscopic cellular detail of the specimen, which can obscure information useful to the diagnosis. Refrigerating specimens in formalin slows fixation.

## VIII. INSTRUCTIONS FOR SPECIFIC POULTRY SAMPLES (SKIN, BONE MARROW, AND KIDNEY)

A. PHVs are to submit poultry skin specimens, approximately 1½-2 x 2 inches, with normal and abnormal areas (preferably, where the two meet). Fixation of skin occurs through the depth or layers of cells of the skin tissue, not across the width of the specimen. For this reason, PHVs may submit skin in larger pieces. If smaller skin specimens are submitted, PHVs are to place the skin on a piece of cardboard to prevent curling. The fat and serum in the skin will adhere it to the cardboard. PHVs are to place the cardboard with the skin attached into the formalin.

B. PHVs are to select bone marrow specimens from the femoral shaft when avian leukosis or osteomyelitis are suspected.

1. For osteomyelitis, submit the distal femur, proximal tibiotarsus, or other bone (usually including the growth plate) in formalin.
2. For avian leukosis, expose the bone and cut a ¾ to 1" portion of the cranial shaft, and crack the bone, but leave bone adherent to marrow. Place the specimen in formalin so that it will preserve adequately.

C. If a kidney specimen is needed, PHVs are to locate the cranial division of the kidney ([Figure 1](#)). PHVs are to make a tangential cut [across the ventrodorsal axis of kidney ([Figure 2](#))] in the cranial division.

Figure 1 Cranial division of the kidney

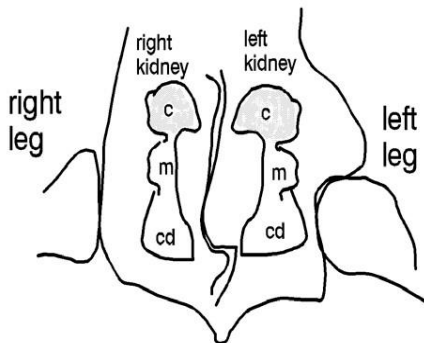
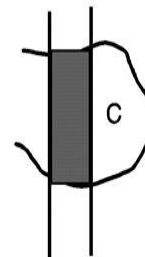


Figure 2 Example of a tangential cut across in the cranial division



## IX. COMPLETION OF FSIS FORM 8000-19 IN PHIS

A. PHVs are to be aware that carbon copy versions of FSIS Form 10,300-2 are no longer available, except at establishments without access to PHIS. PHVs are to complete, submit, and print FSIS Form 8000-19, including the questionnaire, using PHIS. PHVs are to refer to FSIS [Directive 13,000.2](#), *Performing Sampling Tasks in Official Establishments Using the Public Health Information System*, for instructions on submitting pathology samples through PHIS. Chapter XI is specific for poultry establishments and Chapter XII is specific for livestock establishments.

B. PHVs are to submit a separate, completed FSIS Form 8000-19 with the specimens from each individual poultry, livestock, or fish carcass except when pooling tissues as described in [Section IX\(C\)](#). PHVs may use more than one sample jar to submit specimens from a single carcass when needed. PHVs are to affix the same sample identification seal number on each submission jar and the corresponding matching seal on the submission form. This identification is important to maintain the chain of evidence.

C. PHVs are to be aware that each shipping container (yellow pathology box) accommodates two submission jars. PHVs may include specimens from two different carcasses in each sample box. PHVs are to maintain the individual identity of each specimen by utilizing a separate set of laboratory seals for each submission jar and corresponding FSIS Form 8000-19. PHVs are not to pool tissues from different carcasses into one submission jar, except in poultry and fish. PHVs may pool the same tissues, for example liver, for pathologic evaluation of a flock of poultry or lot of fish. PHVs are to include the number of pooled specimens from separate carcasses submitted for each flock of poultry or lot of fish on FSIS Form 8000-19 in this situation. PHVs are to place the tissues in the same jar, provided the tissues meet the fixation requirements in [Section VII\(B\)](#).

D. PHVs are to complete all relevant fields on FSIS Form 8000-19 and include the official establishment number, any retain tag numbers, or other identifying numbers on FSIS Form 8000-19. PHVs are also to complete the questionnaire on FSIS Form 8000-19. The questionnaire may assist the pathologist in providing additional diagnostic information that can be helpful to the PHV when making a disposition. When completing the questionnaire, it is recommended that the PHVs include the following information:

1. Age;
2. Type of tissues submitted;
3. Abnormalities noted during antemortem inspection;
4. Postmortem findings (including carcass condition); and
5. Reason for specimen submission.

E. PHVs are to describe the gross pathology lesions. At a minimum, PHVs are to provide information regarding the lesion's size, color, and consistency and indicate the location on the carcass from which the specimen was taken.

F. PHVs are to include the method of identification used to signify each corresponding lymph node per [Section VII\(B\)\(3\)](#).

## **X. PACKAGING OF TISSUE SPECIMENS FOR SUBMISSION TO THE LABORATORY**

A. PHVs are to follow the directions in FSIS [Directive 7355.1](#), *Use of Sample Seals for Program Samples and Other Applications*, when submitting pathology specimens. The laboratory will discard improperly sealed specimen containers.

B. PHVs are to safeguard the security of tissue specimens while preparing, storing, packaging, and submitting specimens for pathologic evaluation; close all formalin jars tightly to prevent leakage; and do not tape formalin jars closed. PHVs are to include the submission form within the box on the outside of the secondary containment bag in case leakage occurs.

C. PHVs are to send properly prepared and packaged specimen containers via FedEx (overnight billable stamp included in the yellow pathology box) to the:

USDA-FSIS Eastern Laboratory  
Russell Research Center  
950 College Station Road  
Athens, GA 30605

D. PHVs are to send samples collected from cattle suspected of having Tuberculosis (TB) lesions to the National Veterinary Services Laboratory (NVSL) for the Animal and Plant Health Inspection Service. Instructions for requesting TB sampling boxes and submitting TB samples are found in FSIS [Directive 6240.1](#), *Inspection, Sampling, and Disposition of Cattle for Tuberculosis (TB)*. In swine, PHVs are to submit specimens to the NVSL only from animals having generalized thoracic granulomas. PHVs are to complete [VS Form 6-35](#), *Report of Tuberculosis Lesions or Thoracic Granulomas in Regular Kill Animals* and include this form with tuberculosis specimens submitted to the NVSL lab. PHVs are to send samples collected from swine suspected of having TB lesions that do not exhibit generalized thoracic granulomas to the Eastern Laboratory using the FSIS supplied yellow pathology boxes.

## XI. REPORTING OF RESULTS

PHVs are to be aware that the histopathology results will be emailed to the name identified as the collector in PHIS. If the results are to be emailed to other individuals, PHVs are to include those names and email addresses in the collection remarks or in the additional comments section of the sample questionnaire. The PHV is to directly contact the pathologist providing the histopathology results by email or phone if there are questions about the results. PHVs are to send all other inquiries about pathology to the Eastern Laboratory's central email address at [EasternLabPathology@usda.gov](mailto:EasternLabPathology@usda.gov).

## XII. QUESTIONS

Refer questions regarding this directive to your supervisor or to the Office of Policy and Program Development through [askFSIS](#) or by telephone at 1-800-233-3935. When submitting a question, complete the [web form](#) and select **Sampling** for the Inquiry Type.

**NOTE:** Refer to [FSIS Directive 5620.1](#), *Using askFSIS*, for additional information on submitting questions.



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