

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

10230.6

01/01/2015

SUBMITTING TISSUE SPECIMENS FOR PATHOLOGICAL OR DIAGNOSTIC MICROBIOLOGICAL EVALUATION TO THE LABORATORY

I. PURPOSE

The Vermont Agency of Agriculture, Food and Markets (VAAFMM) Meat Inspection Section is issuing this directive to provide Public Health Veterinarians (PHVs) with information on how to submit specimens for pathological evaluation to the NH Veterinary Diagnostic Laboratory. In addition, PHVs may submit tissue samples for diagnostic microbiological evaluation when they submit pathological specimens.

Key Points Covered

- Selection of tissue specimens for pathological evaluation

- Preparation of tissue specimens for submission

- Completion of the Submission Form NHVDL Form 1022-4 and packaging of the specimens

II. REFERENCES

- VT Directive 7355.1
- 6 VSA Chapter 204
- FSIS Regulations 9 CFR 311.1, 381.77, and 381.80
- Federal Meat Inspection Act 21 U.S.C. 604
- Poultry Products Inspection Act 21 U.S.C. 455

III. BACKGROUND

The PHV makes a diagnosis after performing a careful examination and inspection of the carcass and parts. In most cases, once the PHV has made the diagnosis, he or she follows the regulations in making the disposition (i.e., disposal). On rare occasions, PHVs exercise their professional judgment in making the disposition when the regulations do not adequately describe the condition. When necessary, or if the PHV is in doubt of the disposition,

specimens are submitted to assist in making that diagnosis if the carcass is retained, or to confirm a diagnosis if the carcass is already condemned. The PHV combines the organoleptic inspection information with the laboratory information in making a diagnosis.

PHVs may seek diagnostic assistance from a Veterinary Diagnostic Laboratory. A Veterinary Diagnostic Laboratory provides a diagnosis (e.g., metastatic squamous cell carcinoma or malignant lymphoma) or information on the severity and chronicity, as well as etiologic agents observed as a possible cause of a disease condition in animals or carcasses. If PHVs have submitted tissues for a diagnostic microbiological evaluation, the laboratory will only conduct the diagnostic microbiological evaluation if it determines that it is necessary after reviewing the histopathological slides.

IV. SELECTION OF TISSUE SPECIMENS FOR PATHOLOGICAL OR DIAGNOSTIC MICROBIOLOGICAL EVALUATION

PHVs are responsible for the preparation, and the submission to the laboratory, of tissue specimens for pathological or diagnostic microbiological evaluation. PHVs should collect tissue specimens, as necessary, to assist in the disposition determination of a carcass or carcass part. If the PHV has questions concerning the number of carcasses from which specimens should be collected for pathological or diagnostic microbiological evaluation, he or she should seek guidance from the Front-line Supervisor.

A. Where to Collect Tissues for Specimen Submission

Should PHVs decide that it is necessary to collect tissue specimens, they are to follow the instructions below when submitting samples for pathological evaluation.

1. PHVs are to submit tissue specimens from all organs and tissues suspected of having lesions. Whenever possible, PHVs should take tissue sections at a point of transition between normal and pathologic tissues. PHVs should submit potential inflammatory and neoplastic lesions with draining lymph nodes.
2. When systemic conditions are suspected, PHVs are to submit tissue specimens from a representative sample of the major visceral organs (liver, kidneys, spleen, heart, and lungs), two or three lymph nodes draining different areas in livestock, and any gross lesions (abscesses, hemorrhage, necrosis).
3. When neoplasia is suspected, PHVs are to submit tissue specimens from the affected tissue and the surrounding normal tissue. Specimens in livestock should include lymph nodes that have suspected metastatic lesions and lymph nodes receiving lymphatic drainage from the site of origin of the tumor.

4. When Avian leukosis complex is suspected, PHVs are to submit tissue specimens from the skin, sacrosciatic nerve with attached dorsal root ganglion, liver, spleen, kidney, bursa of Fabricius, and any other tissues with suspicious lesions.

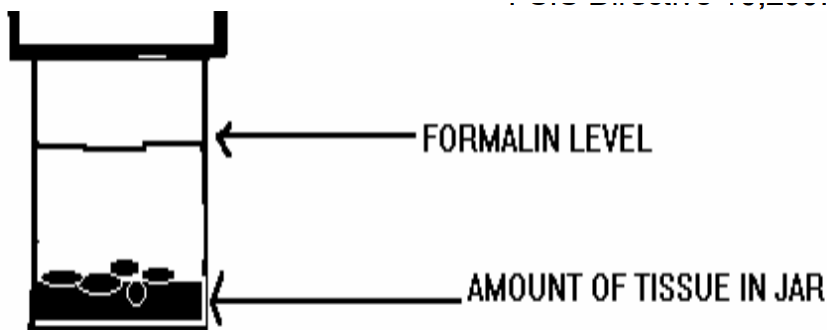
V. PREPARATION OF TISSUE SPECIMENS FOR SUBMISSION

A. Tissue Specimens for Slaughter Pathological Evaluation

Tissue specimens are preserved or fixed for evaluation by placing the tissues in a jar containing formalin kept at room temperature. The formalin will preserve the integrity of the specimens.

1. PHVs are to cut specimens 3/8 inch thick (9 mm). Thicker tissues do not maintain their cellular integrity properly, leading to loss of cellular detail. Avoid prolonged exposure of the tissue specimens to air that results in drying of the tissue surfaces before immersion in formalin. PHVs should not submit intact organs such as lymph nodes or avian spleen without sectioning through the capsule.

2. PHVs are to place tissues immediately in formalin (preserves cellular detail). Prolonged holding, even at refrigerated temperatures, will result in autolytic changes that mimic degeneration. PHVs should use approximately one part tissue to ten parts formalin to adequately preserve or fix the tissue specimen.



NOTE: If formalin is unavailable, PHVs may refrigerate (do not freeze) tissues to retard autolysis until formalin is available.

3. PHVs are to identify lymph nodes or other affected tissues by using metal or plastic devices. For example, PHVs could, when submitting two lymph nodes, place a pink pin in one lymph node and place two staples in the other lymph node for identification purposes. PHVs should use metal or plastic for identification because paper dissolves in formalin. Affected tissue includes the

normal surrounding tissue and any lymph nodes draining lymph from the affected area.

4. PHVs are to store specimens at room temperature. PHVs should not place specimens in the freezer. Freezing tissue reduces the microscopic cell detail of the specimen, and refrigeration of the specimen in formalin retards fixation.

B. Special Instructions for Submitting Poultry Specimens for Slaughter Pathological Evaluation

1. PHVs are to submit skin specimens, approximately 1½-2 x 2 inches, with normal and abnormal areas (preferably, where the two meet). For example, PHVs may submit skin specimens for neoplasia or dermatitis, e.g., squamous cell carcinoma or keratoacanthoma. If smaller skin specimens are submitted, PHVs are to place the skin on a piece of cardboard to prevent curling. The skin will self-adhere to the cardboard due to the fat and serum in the tissue. PHVs should place the cardboard with the skin attached into the formalin. 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, skin may be submitted in larger pieces.

2. PHVs are to select bone marrow specimens from the femoral shaft. For example, PHVs may submit specimens of bone marrow when avian leukosis or osteomyelitis are suspected.

a. For osteomyelitis, PHVs should submit the distal femur, proximal tibiotarsus, or other bone (usually including the growth plate) in formalin.

b. For avian leukosis, PHVs should expose the bone and cut a ¾ to 1" portion of the cranial shaft, and crack the bone, but leave bone adherent to marrow. PHVs should place the specimen in formalin so that it will preserve adequately.

3. PHVs are to take samples the approximate thickness of two stacked nickels; a little thicker sample is acceptable when sampling the kidneys, liver, spleen, intestines, bursa, pancreas, gonads, nerves, substantial skin portions, thymus, and thyroid. It is important not to cut the tissues submitted for pathology in pieces that are too small, since this makes identification of the tissue difficult when preparing tissues for processing. If a kidney specimen is needed, PHVs should make a tangential cut (across longitudinal axis of kidney-see Fig. 2) in the cranial division of the kidney (Fig. 1). PHVs are to include some of the normal tissue around the abnormal tissue when sampling definitive focal lesions (masses), either neoplastic or otherwise.

Fig. 1

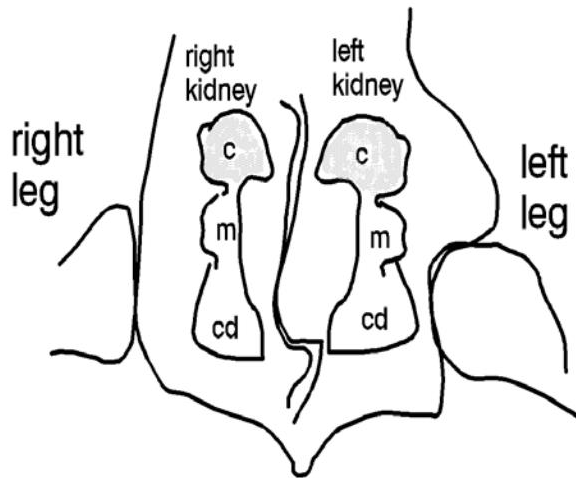
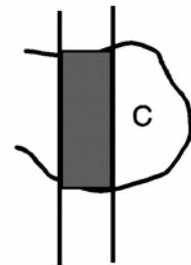


Fig. 2



VI. SUBMISSION OF SPECIMENS

A. Completion of Submission Form NHVDL Form 1022-4

1. PHVs are to submit a separate, completed Submission Form NHVDL Form 1022-4 with each set of specimens from each individual carcass. PHVs should not pool tissues from different carcasses in a jar and may use more than one jar if needed. If PHVs use more than one jar to submit specimens for a specific carcass, the PHV should include on Submission Form NHVDL Form 1022-4 the number of jars submitted associated with a specific carcass. This identification is important to maintain the chain of evidence.

NOTE: In poultry, the same tissue, for example liver, may be submitted for pathological evaluation from a flock. The PHV should include on Submission Form NHVDL Form 1022-4 the number of livers from separate carcasses submitted for each flock. PHVs may place the tissues in the same jar provided they meet the requirements in V A 1-3 above.

2. PHVs are to complete all spaces on Submission Form NHVDL Form 1022-4 that apply.

3. PHVs are to describe the species, sex, age, and condition of the carcass. PHVs are to include information on animal type and origin in the appropriate space. PHVs should accurately describe ante-mortem and post-mortem findings used to determine the diagnosis and indicate why the specimen is being submitted.

4. PHVs are to include some description of gross pathology. It is very

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

5. PHVs are to include the official establishment number, any retain tag numbers, or other identifying numbers on Submission Form NHVDL Form 1022-4.

6. PHVs are to include the method of identification used to signify each coordinating lymph node per V A 3.

7. PHVs are to obtain supplies from the office and most up-to-date forms from the NH Veterinary Diagnostic Laboratory website.

NOTE: PHVs should send samples for suspected Tuberculosis (TB) lesions to the National Veterinary Services Laboratory for the Animal and Plant Health Inspection Service. Instructions for sending TB samples are found in FSIS Directive 6240.1, Bovine Mycobacteriosis Disposition Guideline.

B. Packaging of Tissue Specimens for Submission to the Laboratory

1. PHVs are to follow the directions in VT 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.

2. PHVs are to safeguard the security of tissue specimens during preparation, storing, packaging, and submission of specimens for pathological or diagnostic microbiological evaluation.

3. PHVs are to send properly prepared and packaged specimen containers using the mailing forms provided by the laboratory to the:

NHVDL
21 Botanical Lane
Durham, NH 03824
Phone: 603-862-2726
FAX: 603-862-0179
Email: nh.vdl@unh.edu

PHVs are not to leave specimens under conditions where the specimens in formalin are exposed to freezing temperatures. For example, specimens are not to be left outside for FedEx to pick up when the temperature is below freezing.

Sending specimens to NHVDL

Step 1
Place Samples In Your Choice Of Package

You have the option of using any box, Tyvec bag, or clinpak/overpack you choose. Remember, you can place as many specimens in the package, up to 5 pounds. The more specimens per box saves you money.

Step 2
Apply Courier Label

Apply your preferred courier label.

Step 3
Call Your Courier For Pick Up

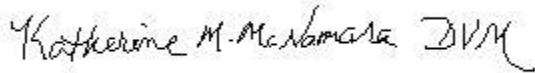
Federal Express: 1 800.GoFedEx (1 800 463-3339)

UPS: 1 800-PICK-UPS (1 800 742-5877)

USPS (US Postal Service)
Place package with your regular mail pick up (or drop off at local post office)

NOTE: PHVs are to record the collection of histopathology tissue specimens in the PHIS.

Questions can be referred to the meat inspection office at 802-828-2426.

A handwritten signature in black ink that reads "Katherine M. McNamara DVM". The signature is written in a cursive style.

Head of Service
VT Meat Inspection Service