TEXAS DEPARTMENT OF STATE HEALTH SERVICES MEAT SAFETY ASSURANCE AUSTIN, TEXAS

MSA DIRECTIVE

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

I. PURPOSE

The Meat Safety Assurance Unit (MSA) is issuing this procedure to provide MSA Veterinarians with information on how to submit specimens for pathological evaluation to the Texas Veterinary Medical Diagnostic Laboratory (TVMDL). In addition, MSA Veterinarians may submit tissue samples for diagnostic microbiological evaluation when they submit pathological specimens.

Key Points Covered

- Selection of tissue specimens for pathological or diagnostic microbiological evaluation
- Preparation of tissue specimens for submission

II. CANCELLATION

MSA Directive 10,230.6

III. RESERVED

IV. REFERENCES

MSA Procedure 7355.1 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

V. BACKGROUND

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

MSA Veterinarians may seek diagnostic assistance from the TVMDL. TVMDL 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 MSA Veterinarians 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.

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

MSA Veterinarians are responsible for the preparation, and the submission to the laboratory, of tissue specimens for pathological or diagnostic microbiological evaluation. MSA Veterinarians should collect tissue specimens, as necessary, to assist in the disposition determination of a carcass or carcass part. If the MSA Veterinarian 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 MSA Central Office, TVMDL, or FSIS Technical Service Center (TSC).

A. Where to Collect Tissues for Specimen Submission

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

1. MSA Veterinarians are to submit tissue specimens from all organs and tissues suspected of having lesions. Whenever possible, MSA Veterinarians should take tissue sections at a point of transition between normal and pathologic tissues. MSA Veterinarians should submit potential inflammatory and neoplastic lesions with draining lymph nodes.

2. When systemic conditions are suspected, MSA Veterinarians 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, MSA Veterinarians 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, MSA Veterinarians 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.

VII. 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. MSA Veterinarians are to <u>cut specimens 3/8 inch thick</u> (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. MSA Veterinarians should not submit intact organs such as lymph nodes or avian spleen without sectioning through the capsule.

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



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

3. MSA Veterinarians are to identify lymph nodes or other affected tissues by using metal or plastic devices. For example, MSA Veterinarians 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. MSA Veterinarians 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. MSA Veterinarians are to store specimens at room temperature. MSA Veterinarians 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.

NOTE: MSA Veterinarians with access to a microscope may request histologic slide preparations of tissues from a submitted case. Review of the slides may be used for their own professional development. However, the MSA Veterinarians interpretation

of the histologic preparations cannot be used by itself to make a determination of the disposition of the carcass.

B. Tissue Specimens for Diagnostic Microbiological Evaluation

If a MSA Veterinarian decides to request the diagnostic microbiological evaluation of tissue samples in conjunction with the pathological evaluation, he or she should submit solid tissues that are frozen. MSA Veterinarians should submit exudates or other tissue fluids using a sterile culturette for culture.

MSA Veterinarians are to prepare frozen samples for submission when requesting diagnostic microbiological evaluation in addition to the preserved tissue samples. The samples for microbiological examination are **not** placed in formalin. Submit samples as either:

1. culturettes of affected tissues placed in the slot in the foam padding of standard pathology containers; or

2. tissues in leak-proof bags, frozen, and placed in a separate special frozen product container for shipping.

NOTE: Culturettes are plastic tubes that pull apart. The top half has an attached sterile cotton swab. The bottom half contains a liquid transport medium in a glass vial. MSA Veterinarians are to swab the affected site and then place the swab firmly into the bottom half of the plastic tube to close. MSA Veterinarians then should crush the bottom of the plastic tube containing the liquid-filled pellet to maintain a moist environment during transit. Culturettes are available from the pathology laboratory upon request.

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

1. MSA Veterinarians are to submit skin specimens, approximately $1\frac{1}{2}$ -2 x 2 inches, with normal and abnormal areas (preferably, where the two meet). For example, MSA Veterinarians may submit skin specimens for neoplasia or dermatitis, e.g., squamous cell carcinoma or keratoacanthoma.

NOTE: If smaller skin specimens are submitted, MSA Veterinarians 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. MSA Veterinarians 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. MSA Veterinarians are to select bone marrow specimens from the femoral shaft. For example, MSA Veterinarians may submit specimens of bone marrow when avian leukosis or osteomyelitis are suspected.

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

b. For avian leukosis, MSA Veterinarians should expose the bone and cut a 3/4 to 1" portion of the cranial shaft, and crack the bone, but leave bone adherent to marrow. MSA Veterinarians should place the specimen in formalin so that it will preserve adequately.

3. MSA Veterinarians 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, MSA Veterinarians should make a tangential cut (across longitudinal axis of kidney-see Fig. 2) in the cranial division of the kidney (Fig. 1). MSA Veterinarians are to include some of the normal tissue around the abnormal tissue when sampling definitive focal lesions (masses), either neoplastic or otherwise.

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VIII. SUBMISSION OF SPECIMENS

A. Completion of TVMDL Submission Form

1. MSA Veterinarians are to submit a separate, completed TVMDL Submission Form with each set of specimens from each individual carcass. MSA Veterinarians should not pool tissues from different carcasses in a jar and may use more than one jar if needed. If MSA Veterinarians use more than one jar to submit specimens for a specific carcass, the MSA Veterinarians should include on the TVMDL Submission Form 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 MSA Veterinarian should include on the TVMDL Submission Form the number of livers from separate carcasses submitted

for each flock. MSA Veterinarians may place the tissues in the same jar provided they meet the requirements in VII A 1-3 above.

2. MSA Veterinarians are to complete all spaces on the TVMDL Submission Form that apply.

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

4. MSA Veterinarians are to include some description of gross pathology. It is very important to describe lesions. At a minimum, MSA Veterinarians 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. MSA Veterinarians are to include the official establishment number, any retain tag numbers, or other identifying numbers on the TVMDL Submission Form. MSA Veterinarians should indicate the preferred method for reporting results (phone, e-mail, or fax). For e-mail, MSA Veterinarians are to clearly print their name as it appears in the Outlook address book list. MSA Veterinarians should use only fax numbers to a dedicated government fax machine.

6. MSA Veterinarians are to include a separate TVMDL Submission Form for any microbiological samples when placed in a separate special frozen product container. MSA Veterinarians should correlate diagnostic microbiological samples to the formalin-fixed tissues submitted. On the form for the microbiological sample, MSA Veterinarians are to state that a corresponding formalin-fixed tissue specimen was sent to the laboratory. On the formalin-fixed tissue specimen form, MSA Veterinarians are to identify the respective frozen sample that was sent to the laboratory. This information is needed to maintain the chain of evidence.

7. MSA Veterinarians are to obtain supplies and forms from the TVMDL.

NOTE: MSA Veterinarians 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. MSA Veterinarians are to follow the directions provided by TVMDL in accordance with Department of Transportation requirements and regulations. MSA seals should be used appropriately when packing samples to maintain the chain of evidence.

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

3. Samples are to be properly prepared and packaged in approved specimen containers via UPS (or other DSHS approved carrier). MSA Veterinarians should send the sample to the nearest TVMDL location from the below list.

Canyon Laboratory	College Station Laboratory
Courier/GPS Address	Courier/GPS Address
3209 Russell Long Blvd.	483 Agronomy Rd
Canyon, TX 79016	College Station, TX 77843-4471
USPS Mailing Address	USPS Mailing Address
WT Box 60818	PO Drawer 3040
Canyon, TX 79016	College Station, TX 77841-3040

NOTE: MSA Veterinarians 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 UPS (or other DSHS approved carrier) to pick up when the temperature is below freezing.

IX. QUESTIONS

Refer questions through supervisory channels.

James R. Dillon

James R. Dillon, DVM, MPH Director, Texas State Meat and Poultry Inspection Program Department of State Health Services