INSPECTION, SAMPLING, AND DISPOSITION OF CATTLE FOR TUBERCULOSIS (TB)

I. PURPOSE

FSIS is reissuing this directive with updated instructions to inspection program personnel (IPP) and Public Health Veterinarians (PHVs) regarding the inspection, sampling, and disposition of cattle identified by the Animal and Plant Health Inspection Service (APHIS) because of TB, or regular cattle found to have TB lesions on post-mortem. This directive provides instructions for IPP to verify that the establishment collects and maintains identification (ID) of livestock during slaughter. This directive provides PHVs the option of assigning and observing IPP performing routine inspection procedures on restricted TB Exposed, Category 2 cattle (defined below). This directive includes instructions on how IPP are to document slaughter of bovine with TB in the Public Health Information System (PHIS).

KEYPOINTS:

- The instructions in this directive are for IPP at official establishments that slaughter cattle
- IPP are to verify that the establishment collects animal ID needed to accurately trace back the origin of cattle with TB
- When large numbers of TB exposed cattle, Category 2 only (defined below), are presented to the PHV for ante-mortem inspection and passed for slaughter, under certain conditions, PHVs may direct IPP to perform required inspections and further inspect the tissues incised by IPP on-line at a reduced speed

II. CANCELLATION

FSIS Directive 6240.1, Rev. 1, Inspection, Sampling, and Disposition of Animals for Tuberculosis, 1/29/09

III. BACKGROUND

A. Cattle TB is an infectious and communicable granulomatous disease caused by Mycobacterium bovis (M. bovis). Through USDA surveillance and eradication efforts, TB is an increasingly rare microbial disease in domestic livestock but remains as a public health concern. The current status of TB eradication efforts in domestic bovine is listed here. Livestock, humans, and wildlife are susceptible at varying degrees to TB caused by M. bovis, M. tuberculosis, or M. avium. M. avium is nearly ubiquitous in the environment and can be isolated from soil and water.

B. TB is spread by direct contact, inhalation of infected droplets expelled from infected lungs (or granulomatous lesions), and ingestion of contaminated feed or milk. TB lesions in livestock and wildlife vary in their appearance and ability to form granulomas based on the host, etiological agent, route of transmission (e.g. aerosol vs. ingestion), duration of infection, and host immunity. Chronic lesions from systemic tuberculosis can be found anywhere in the carcass.

DISTRIBUTION: Electronic OPI: OPPD
C. APHIS bovine TB surveillance efforts focus primarily on identifying new sources of TB from \textit{M. bovis} in bovines. To supplement \textit{APHIS' Cattle Health Monitoring Surveillance} program, APHIS relies greatly on the inspection efforts of FSIS IPP at federally inspected slaughter establishments to detect, retain, and submit granulomatous lesions suggestive of TB from cattle carcasses. FSIS has signed a Memorandum of Understanding (MOU) with APHIS regarding the collection of specimens to achieve national surveillance for the National Tuberculosis Eradication Program. To ensure a minimum level of sampling for national surveillance, APHIS has set a goal for IPP to collect a minimum of one granulomatous or other atypical lesion suggestive of TB per 2,000 normal unrestricted adult cattle (i.e., cows/bulls). IPP are also to submit any granulomatous lesions from any cattle to the National Veterinary Services Laboratories (NVSL) in Ames, Iowa, or to another laboratory officially approved by VS. See APHIS' \textit{Cattle Tuberculosis Disease Information} page.

D. \textit{9 CFR 77.17} outlines requirements applicable to the interstate movement of TB reactor, suspect, or exposed cattle and bison and the official means of identifying them.

E. \textit{9 CFR 310.2} requires that an establishment collect all man-made ID devices from livestock. IPP verify that the establishment collects all live animal ID for purposes of residue and reportable disease trace back. To pinpoint the origin of new cases, NVSL may conduct DNA testing on tissue samples submitted for TB testing.

F. Any TB submission by FSIS IPP that identifies a new case of TB is eligible for TB slaughter surveillance awards funded by APHIS-Veterinary Services (VS).

IV. APHIS CLASSIFICATIONS OF CATTLE WHOSE MOVEMENT IS RESTRICTED FOR TB

A. Live cattle whose movement is restricted by APHIS for TB are identified by APHIS as 1) TB reactors, 2) TB suspects, and 3) TB exposed. All cattle whose movement is restricted by APHIS because of TB are accompanied by APHIS VS Form 1-27.

B. APHIS defines TB reactors, suspects, and exposed cattle in APHIS' \textit{Cattle TB Eradication Uniform Methods and Rules (UMR)}. The APHIS' TB suspect designation is distinct from FSIS' U. S. Suspect designation in \textit{9 CFR 309.18}.

C. APHIS-VS classifies as “exposed” any cattle based on their association with livestock in which \textit{M. bovis} has been diagnosed. TB exposed cattle are subdivided into the following categories:

1. Category 1: cattle that have been moved from an infected herd before the time the infection was disclosed, but after the herd apparently became infected. When traced, these animals are critical for establishing the disease status of the receiving herd; or

2. Category 2: cattle that are part of a known affected herd that test negative or are untested. These cattle may move to slaughter as routine culls of individual animals or by entire herd. If the TB exposed category of restricted cattle is not specified, IPP are to handle such cattle as under Category 2.
V. ADDITIONAL CONSIDERATIONS RELATIVE TO COLLECTION OF MAN-MADE ID DEVICES (9 CFR 310.2) AND INSPECTION

A. IPP are to verify that the establishment collects and handles all man-made animal ID, foreign and domestic, present on each animal at ante-mortem (AM). IPP are to also verify that the establishment maintains such ID until post-mortem (PM) inspection is completed per 9 CFR 310.2. For additional instructions, IPP are to refer to the following issuances regarding collection of animal ID:

1. FSIS Directive 6100.1, Ante-mortem Livestock Inspection
2. FSIS Directive 6100.2, Post-mortem Livestock Inspection
3. AskFSIS Q&A on Animal ID
4. Types of Official Animal ID

B. In cattle slaughter establishments, the PHV is to assess, review, and discuss the impact of the instructions in this directive with the establishment within 30 days of issuance, including whether:

1. The establishment is effectively collecting all man-made animal ID on all cattle;
2. All such collected ID from each carcass can accurately be attributed to the corresponding carcass until PM inspection is complete;
3. All such collected ID from each retained carcass is made available to the PHV upon request;
4. Animal ID is collected in a manner that preserves the integrity of DNA identifying the affected cattle. The animal ID is:
   a. Removed from the carcass with a dime sized piece of hide attached to the tag when submitted with the sample;
   b. Removed with attached hair (including the hair roots) remaining on back tags when submitted to the lab;
   c. Collected in a manner to minimize contamination with blood from more than one animal; and
   d. Not cleaned.
5. The routine inspection procedures of large numbers of TB exposed, Category 2 cattle may be performed on-line and at a reduced speed as determined by the PHV.
6. PHV may also need assistance in handling and tracking restricted cattle condemned on ante-mortem as non-ambulatory disabled (NAD) or dead. Arrangements for a necropsy of such restricted cattle are required.

VI. ANTE-MORTEM INSPECTION OF RESTRICTED CATTLE

A. When the PHV receives notification from the establishment of the arrival of any restricted cattle identified as TB reactors, TB suspects, or TB exposed on APHIS Form VS Form 1-27, the PHV is to verify
that the establishment segregates each lot of restricted cattle from all other animals and identifies them with their respective category (e.g. TB reactor, suspect, exposed).

B. The PHV is to coordinate with the establishment to arrange the orderly slaughter and thorough inspection of all restricted cattle presented for AM inspection. The PHV is to verify the handling of restricted cattle as provided in 9 CFR 311.2 and this directive.

C. When the PHV receives notification from the establishment of the arrival of TB reactors identified on APHIS Form VS Form 1-27, the PHV is to:

1. Perform a complete physical examination of the cattle, including taking their temperatures, and record the examination results on FSIS Form 6150-1, Identification Tag – Ante mortem;

2. Indicate to the establishment that restricted cattle passed for slaughter are to be handled on the slaughter floor the same way as any U. S. Suspect. Note: The TB “USDA Reactors” tag is used in lieu of a U. S. Suspect tag;

3. Record the reactor tag number or official metal ear tag number on the FSIS Form 6150-1, Identification Tag – Ante mortem for cattle bearing official “USDA Reactor” ear tags.

NOTE: APHIS VS or State personnel may accompany TB suspect cattle to slaughter and provide additional guidance regarding the ID and status of particular animals prior to inspection.

D. When the PHV receives notification from the establishment that TB reactor, TB suspect, or TB exposed cattle have arrived dead (DOA) at the establishment, died in the pens (DIP), or are condemned at AM inspection, the PHV is to:

1. Perform the routine inspection procedures described in FSIS Directive 6100.2, Post- Mortem Livestock Inspection and the expanded post-mortem (EPM) inspection procedure described in Guideline No. 4, “Inspection of Tuberculin Reactors,” and summarized in Attachment 1 of this directive; and

2. Submit tissue from all granulomatous lesions identified during the EPM inspection procedures regardless of their anatomical location per this directive.

NOTE: If the establishment does not have adequate inedible facilities to examine carcasses of restricted cattle condemned on AM by the PHV, the PHV is to request assistance from APHIS. In response to the request, qualified available APHIS personnel may assist the PHV with correlations of ID devices, collecting and submitting tissues, performing on-site necropsy or PM examination, or finding a suitable location off premises to collect TB samples from cattle not passed for slaughter.

E. Upon arrival of restricted cattle identified on VS Form 1-27 that are passed for slaughter, the PHV is to coordinate with the establishment, arrange the approximate time of slaughter, and alert IPP to their approximate arrival at the slaughter floor.

F. When cattle with official TB ID (e.g. tuberculin brand or ear tag) without the proper accompanying paperwork identifying the animal (i.e. VS 1-27) or restricted cattle as identified on the VS 1-27 without official TB ID are presented for AM inspection, the PHV is to:

1. Verify that the establishment has segregated the unreconcilable, misidentified, or unidentified restricted cattle from other livestock;

2. Maintain regulatory control of such cattle; and
3. Withhold such animals from slaughter until the PHV receives further instructions from APHIS.

**NOTE:** Regulatory control may entail the use of chain and pad lock or US Reject tag (FSIS Form 6502-1) on the pen gate or a US Condemned ear tag on the animal.

**VII. CONTACTING APHIS**

A. For questions regarding the unexpected arrival of restricted cattle at an establishment, PHVs can contact the District Office, the APHIS Assistant District Director (ADD) (formerly Area Veterinarian-in-Charge (AVIC)), Service Center Director (SCD), or designee for further instructions.


B. For questions regarding sampling supplies or collection and submission to APHIS- NVSL

   USDA APHIS  
   National Veterinary Services Laboratories  
   1920 Dayton Avenue  
   Ames, Iowa 50010  
   515-337-7212

**VIII. IPP PERSONAL SAFETY**

A. IPP are reminded to follow all standard sanitary procedures and good hygienic practices for maintaining personal equipment and hygiene after detecting or handling tissues with granulomatous lesions. IPP are to verify establishment employees follow the same procedures and practices as determined by the PHV.

B. If not already on file, IPP can obtain Material Safety Data Sheets (MSDS) for 10% neutral buffered formalin and saturated sodium borate solutions from the APHIS or vendor web sites.

**IX. POST-MORTEM INSPECTION AND DISPOSITION PROCEDURES**

A. The PHV is to coordinate and arrange with the establishment the orderly slaughter and thorough inspection of all restricted cattle passed for slaughter. The PHV is to make all dispositions of cattle with TB based on requirements in 9 CFR 311.2.

**NOTE:** If APHIS VS has not identified the category of the TB exposed cattle as Category 1 or Category 2 on VS Form 1-27, then IPP are to handle the TB exposed cattle as Category 2 at PM inspection.

B. For TB reactors at PM or any necropsy performed on TB restricted cattle (i.e. TB reactor, suspect, or exposed) condemned on AM (e.g. DOA, DIP), the PHV is to:

   1. Perform the EPM inspection procedures described in the *Guideline No. 4, “Inspection of Tuberculin reactors”* and outlined in Attachment 1 of this directive in addition to regular PM inspection procedures in *FSIS Directive 6100.2, Post-Mortem Livestock Inspection*;

   2. When no granulomas or suspicious lesions are found in TB reactors, submit a representative sample of lymph nodes of the head and thorax for histopathology and bacteriologic examination to NVSL. Be aware microbiological samples must get to NVSL as quickly as possible as delays reduce the chances of recovery when organism numbers are low.
C. For TB Suspect and TB Exposed – Category 1 cattle, the PHV is to perform the modified expanded PM (MEPM) procedures by incising the supramammary and mesenteric lymph nodes in addition to the regular post-mortem inspection procedures in FSIS Directive 6100.2, Post-Mortem Livestock Inspection. See the description of procedures in the Guideline No. 4, “Inspection of Tuberculin reactors” and summary of MEPM in Attachment 1.

NOTE: APHIS or State officials may also request the submission of representative samples of lymph nodes of the head and thorax from suspect or exposed cattle without granulomatous lesions, either on the APHIS VS Form 1-27 or via officials accompanying the animals. Generally, such sampling is limited to 4 animals in a shipment. The PHV should try to accommodate such requests whenever possible.

D. For TB Exposed - Category 2 cattle, the PHV is to perform the routine PM inspection procedures per FSIS Directive 6100.2, Post-Mortem Livestock Inspection. With FLS approval based on adequate facilities to ensure IPP safety, PHVs may direct IPP to perform, observe, and further inspect the routine PM inspection procedures performed on these cattle. Such inspections may be performed at a reduced speed as determined by the PHV per 9 CFR 310.1(b)(1).

NOTE: Per 9 CFR 310.1(b)(1), the PHV has the authority to require the establishment to reduce slaughter line speeds where, in his judgment, the inspection procedure cannot be adequately performed at the current line speed because the health condition of the particular animals indicates a need for more extensive inspection. Proper coordination with the establishment and IPP will keep unnecessary delays to a minimum.

E. For all cattle (i.e. restricted or not) found with granulomatous lesions on PM, the PHV is to:

1. Perform the EPM inspection procedures described in the Guideline No. 4, “Inspection of Tuberculin reactors” and outlined in Attachment 1 in this directive in addition to regular PM inspection procedures in FSIS Directive 6100.2, Post-Mortem Livestock Inspection, of this directive;

2. Record observations of all granulomatous lesions into the Animal Disposition Reporting (ADR) in PHIS. For each sample submission, enter the animal ID information and cross-reference the laboratory sample submission form number in the narrative text box under the PHIS-ADR menu. See Figure 6 below;

3. Submit all granulomatous lesions to the NVSL per sampling instructions in Part IX below;

4. Retain the carcass whose disposition is pending laboratory results. See Documentation in Part XI below.

5. Use professional judgment and laboratory results in making the appropriate final presumptive diagnosis based on gross pathology, stage of the disease, the overall condition of the carcass;

6. Consider a histopathology result from NVSL, “Compatible with mycobacteriosis” as positive for M. bovis. The PHV is to base the final carcass disposition for TB on these histopathological findings and their observations; and

NOTE: The PHV is to make final dispositions on retained carcasses found with conditions other than TB based on laboratory results (e.g. malignant lymphoma) and his or her gross post-mortem observations per 9 CFR 311.

7. Verify proper disposal of the carcass and parts identified as positive for M. bovis in accordance with 9 CFR 311.2. Establishments may elect to condemn carcasses and parts passed for cooking.
X. TISSUE SAMPLING

A. Regardless of a cattle’s age or TB status classification, IPP are to submit:

1. All head and thoracic granulomas where TB lesions are most common, and

2. Any other granulomatous lesions suggestive of TB regardless of the anatomical location. PHVs can find examples of these types of lesions and additional information in:

   a. The PHV training material titled: *Multi-species Disposition Basics with a Public Health Focus*. Search for “tuberculosis”;

   b. FSIS Guideline No. 4, Inspection of Tuberculin reactors; and


3. Representative samples of lymph nodes of the head and thorax of TB reactors when no lesions were observed.

B. Having already submitted any and all granulomatous lesions where TB is suspected, the PHV is to also collect possible lesions or abnormal tissues not typically associated with TB from any adult dairy or beef cows or bulls to ensure a sampling at a minimum rate of one lesion per 2,000 head. IPP are to also submit any granulomatous lesions from steers and heifers as they occur. IPP are not to repeatedly submit samples that are clearly not TB (e.g. malignant lymphoma or large fungal granulomas in GI tract).

C. The PHV is to use the following procedures to prepare tissues for sampling:

1. Remove excess fat;

2. For histopathology, include normal tissue and cut into sections approximately 1 cm (¼ inch) thick and place in a 10% neutral buffered solution (pH 7.0) formalin at a 1:10 tissue to preservative (v/v) ratio. The PHV is to make the 1:10 estimate through visualization;

3. For bacteriologic examination, cut a section approximately 1 to 2 inches thick. Place section in a sodium borate solution at a 1:1 tissue to preservative (v/v) ratio. Make the 1:1 (v/v) estimate visually. Unlike the formalin fixed sample, do not to make additional incisions into the sample because sodium borate is bactericidal;

   **NOTE:** Sodium borate solution is a supersaturated solution. It is normal to see crystals in the bottles containing the solution.

4. If not enough tissue is available to divide for both histopathological and bacteriologic analyses, send the entire tissue sample for histopathological examination; and

5. Using a water-proof ink pen, write on the labels of the specimen bottles the:

   a. Serial number of the VS Form 6-35 or VS Form 10-4:

   b. Ear tag number,

   c. NVSL-supplied label number, or

   d. “U.S. Retained” tag number.
XI. SAMPLE FORMS AND SUBMISSION

A. When submitting granulomatous lesion specimens from regular cattle (i.e. not under restriction as TB reactors, TB Suspects, or TB Exposed), the PHV is to:

1. Complete blocks 1-21 of VS Form 6-35, Report of Tuberculosis Lesions or Thoracic Granulomas in Regular Kill Animals, including all available information for trace back purposes;

2. Include a telephone number in block 18 if the PHV retains the carcass pending laboratory results;

3. Place two copies of the form in the black and yellow striped mailer in the polystyrene box containing the specimens (See Figure 1);

4. Retain the third copy; and

5. See Guidebook for completing VS Form 6-35 for additional information.

B. When submitting TB samples from cattle slaughtered under restriction (i.e. TB Exposed, TB Suspect, or TB reactor cattle received under VS 1-27 permit), IPP are to use VS Form 10-4, Specimen Submission, available electronically.

C. For each sample submission, the PHV is to:

1. Use the pre-paid shipping (e. g. Federal Express) labels, sample vials, plastic bags, and the appropriate form provided by the NVSL in the black and yellow striped mailer box;

2. Secure all the corresponding unwashed man-made non-FSIS ID devices to NVSL in the enclosed plastic bag. If cattle have no tags, then the PHV is to include a description of the cattle on the sample submission form or as an attachment;
3. Include the completed **VS Form 6-35** for each TB specimen from cattle slaughtered under regular inspection (i.e. without restriction) or completed **VS Form 10-4** with TB specimens from restricted cattle (i.e. reactors, suspects, and exposed cattle animals);

4. Submit only one reporting form for tissues from any single cattle to avoid the possibility of the laboratory assigning more than one laboratory accession number to a single animal;

5. Include the specimens and identify the sample vial label and contents of each vial on the accompanying report; and

6. Send the collected tissue samples in formalin and in sodium borate solution, the corresponding animal ID, and completed sample form using the striped mailer box to:

   USDA APHIS  
   National Veterinary Services Laboratories  
   1920 Dayton Avenue  
   Ames, Iowa 50010  
   515-337-7212

**NOTE:** The NVSL will return boxes with supplies after receiving the samples. If a PHV needs more boxes or sampling supplies, the PHV can contact the NVSL or the VS ADD or SCD.

### XII. DOCUMENTATION OF LABORATORY SAMPLES AND DISPOSITION IN PHIS

A. IPP are to record the collection of each TB sample in PHIS as follows:

1. Use the Animal Disposition menu to access “Establishment Reporting” submenu;

2. After selecting the appropriate establishment, date, shift, and type, select “Add Inspected Slaughter” under the “Class Summary List” view (Figure 2);

3. After entering the class (cattle) and sub-class (e.g. beef cow), the link to “APHIS Sampling” is visible (Figure 4); and

4. Enter the number of TB samples and related information (Figure 5) under “Comment.”

   ![Figure 2 - Adding Inspected Slaughter](image)

B. NVSL will report histopathology results by telephone, fax, e-mail, or regular mail to the appropriate in-plant IPP within 24-48 hours after receiving the sample. The PHV may call NVSL if no results are made available after 72 hrs. The PHV is to base the carcass disposition on histopathology findings and his or her observations.
Figure 3 - Entering Sub-Class data

Figure 4 - Link to APHIS Sampling appears in Class Summary Information Page after entry of Sub-class

C. For your information, NVSL will report mycobacterial culture and PCR results separately to the appropriate APHIS Veterinary Services staff only. These results are reported weeks later and do not influence the disposition of the carcass.

Figure 5 - Recording the number of TB samples in PHIS.

D. IPP are to document relevant animal ID, MPD Retain Tag numbers, and final disposition in the “Daily Disposition Record Detail” in PHIS (Figure 6 below).
XIII. COMPLETING APHIS FORM VS 1-27

VS Form 1-27, Permit for Movement of Restricted Animals, accompanies TB reactor, suspect, and exposed cattle to slaughter. The PHV is to complete blocks 26-34 on VS Form 1-27 and distribute copies per instructions listed on the form.

XIV. DATA ANALYSIS

OPPD-Policy Development Staff will contact APHIS-VS-Cattle Health Center and NVSL to review trends in sampling every two years upon issuance of this directive.

XV. QUESTIONS

Refer questions regarding this directive to the Policy Development Staff through askFSIS or by telephone at 1-800-233-3935. When submitting a question, use the Submit a Question tab, and enter the following information in the fields provided:

Subject Field: Enter Directive 6240.1
Question Field: Enter question with as much detail as possible
Queue Field: Select “Slaughter” from drop down menu
Product Field: Select “General Inspection Policy” from the drop down menu
Category Field: Select “Slaughter – Livestock” from the drop down menu
Policy Arena: Select “Domestic” from the drop down menu

When all fields are complete, press Continue and at the next screen press Finish Submitting Question.

NOTE: Refer to FSIS Directive 5620.1, Using askFSIS, for additional information on submitting questions.
### Table 1 - Comparison and Application of Routine, Expanded, and Modified Expanded PM inspection procedures in Cattle

<table>
<thead>
<tr>
<th>Cattle TB Status</th>
<th>Post Mortem Procedures Performed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Restricted or Unrestricted with New Lesions</strong></td>
<td><strong>Routine PM inspection procedures with PHV in attendance.</strong></td>
</tr>
<tr>
<td>TB Exposed - Category 2</td>
<td>TB reactor, or any cattle found with TB lesions at PM ---OR--- All TB Restricted Cattle (TB reactor, suspect, or exposed) condemned on AM (e.g. DOA, DIP)</td>
</tr>
</tbody>
</table>

**Post Mortem Procedures Performed**

- **Routine PM inspection procedures** with PHV in attendance.
- **Modified Expanded Post Mortem (MEPM)** + routine inspection procedures.
- **Expanded Post Mortem (EPM) Inspection Procedures in MPI Guideline No. 4, Inspection of Tuberculin reactors + routine inspection procedures.**

**HEADS**

- See FSIS Directive 6100.2

**VISCERA – PLUCK**

- Left and Right Tracheobronchial
  - See FSIS Directive 6100.2.
- Lungs
  - Palpate + INCISE

**VISCERA – GUTS**

- Cr. and Cd. Mesenteric Ln.
  - See FSIS Directive 6100.2.
- Hepatic (Portal) Ln.
- Liver
  - Palpate + INCISE body of LIVER
- Spleen
  - +PALPATE
- Ovaries, Oviduct, and Uterus
  - +SPECIFICALLY OBSERVE

**CARCASS**

- Deep Popliteal Ln.
  - See FSIS Directive 6100.2.
- Subiliac (Pre-femoral) Ln.
  - +INCISE
- Mammary Ln.
  - (Supramammary or Superficial Inguinal Ln.)
  - +INCISE
- Medial (Internal) Iliac Ln.
  - +INCISE
- Superficial Cervical Ln.
  - +INCISE
- Cd. Deep Cervical Ln.
  - +INCISE
- Cr. and Middle Deep Cervical Ln.
  - +Observe and (optionally) INCISE
| Parietal Pleura |  | +PALPATE |