Tuberculosis Sample Submission Manual for Meat Inspection Personnel

USDA APHIS VS SPRS
Cattle Health Center
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Introduction

The Cooperative State-Federal Bovine Tuberculosis Eradication (TB) program began in 1917. At that time, approximately five percent of the cattle in the U.S. were infected with TB. For many years, the main focus of the program was the area testing of all cattle with the subsequent slaughter of reactor animals. The program proved to be successful as evidenced by the fact that by 2013 the prevalence rate of the disease had decreased to approximately .0008 percent, or less than one infected cattle herd per 100,000 herds in the U.S. At that time, due to the low prevalence rate, continuing the test and slaughter practice was no longer found to be an effective way of detecting new cases of the disease and emphasis shifted to detecting TB at slaughter.

Today, slaughter surveillance continues to be the primary means of detecting bovine TB. In recent years, greater than 80 percent of the TB-infected herds have been detected through slaughter traceback and subsequent epidemiological investigations. The success of the TB eradication program depends to a large degree on the efforts of the State and Federal meat inspectors. Accurate tissue selection and carcass identification leads to the prompt identification of affected herds. The information provided to APHIS through these slaughter submissions allows APHIS's Veterinary Medical Officers (VMO) to effectively investigate herds that are thought to be infected with bovine tuberculosis.

Submitting lesions resembling tuberculosis to the National Veterinary Services Laboratories (NVSL) in Ames, Iowa, is important for the following reasons:

1. Sample submission leads to finding newly infected animals and herds; and

2. As the level of TB further decreases, it becomes increasingly important to have an adequate number of granulomatous lesions submitted to effectively detect an affected herd; and

3. The information gained by conducting TB surveillance can be used to show our trading partners that the U.S. has a very low prevalence level of this disease.

The photographs on the following pages are presented as an aid for State and Federal meat inspection personnel in detecting suspicious lesions for submission to NVSL. They illustrate similarities as seen on the kill floor between TB lesions and those caused by other disease processes. These photographs of lesions were taken in the slaughter plant, first in the viscera tray on the kill floor and then close up in the inspector's office when preparing lesions for submission.

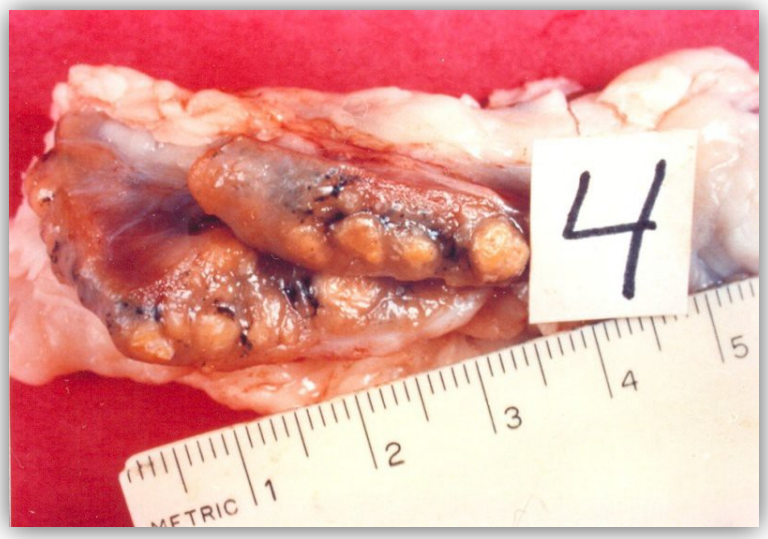
All photographed lesions were confirmed to be caused by *M. bovis*. Lesions were obtained from several animals during the evaluation of a herd of which 45 reactor animals had the following distribution of tuberculous lesions:

Head Only	3
Thoracic Only	15
Abdominal Only	4
Head and Thorax	6
Head and Abdomen	7
Head, Thorax, and Abdomen	8

This data illustrates that the thorax and associated respiratory lymph nodes are not the only sites where tuberculosis lesions may be found. Bovine tuberculosis can cause serious disease in both young and old cattle. Older cattle may have had a longer overall exposure to tuberculosis, but calves can become infected in the uterus or by drinking milk from a tuberculous cow and may develop lesions as well. During federal fiscal years 2010-2014, 24 percent of cattle with TB lesions were dairy and beef cows and bulls, and 76 percent were fed cattle (steers and heifers). Most fed cattle with TB lesions were imported.

This manual is prepared by APHIS as a guide for identifying and preparing tissue samples for submission to NVSL. The first section gives pictorial examples of common tuberculous lesions, while the remainder of the manual is intended as a handbook for the proper submission of suspect granulomatous lesions. It must be stressed that tuberculous lesions can grossly resemble lesions caused by other disease processes. Therefore, all granulomas should be considered suspect for tuberculosis and submitted to NVSL for confirmation.

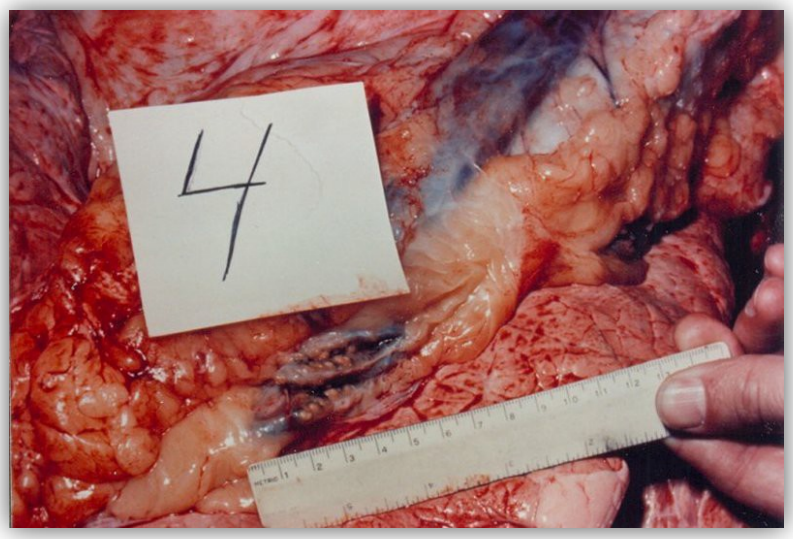
Atlas of Tuberculosis and Tuberculosis-Like Lesions



Lesion 1

Lesion 1 - Bronchial Lymph Node

Tubercles (small, spherical, tan, and firm nodules) - These one millimeter to 20 millimeter lesions are those typically seen when nothing is obscuring the lesions, the ideal situation.



Lesion 2

Lesion 2 - Bronchial Lymph Node
The discreet lesions are the typically yellow or tan, when not hidden by adjacent tissue or hemorrhage. Some of the adjacent tubercles have coalesced into larger masses.

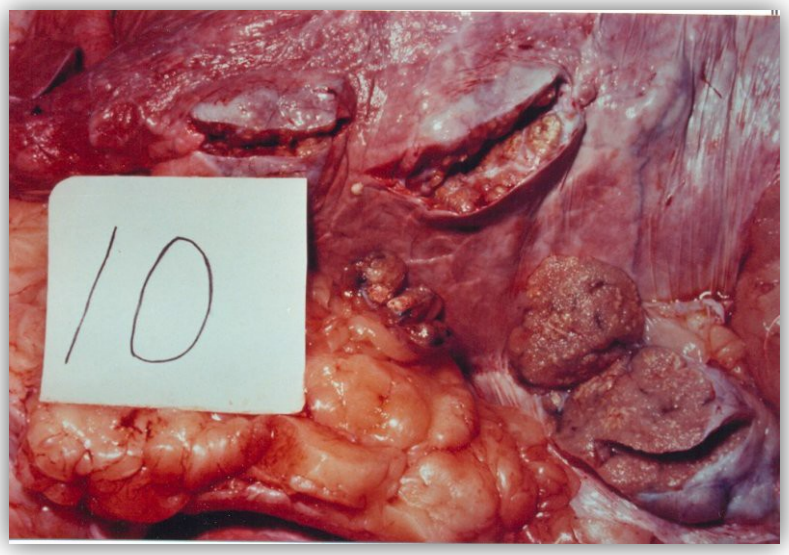
Lesion 3

Lesion 3 - Bronchial Lymph Node
Incised node with several discreet tubercles - These may look similar to abscesses.

Lesion 4

Lesion 4 - Lymph Node with Tubercles

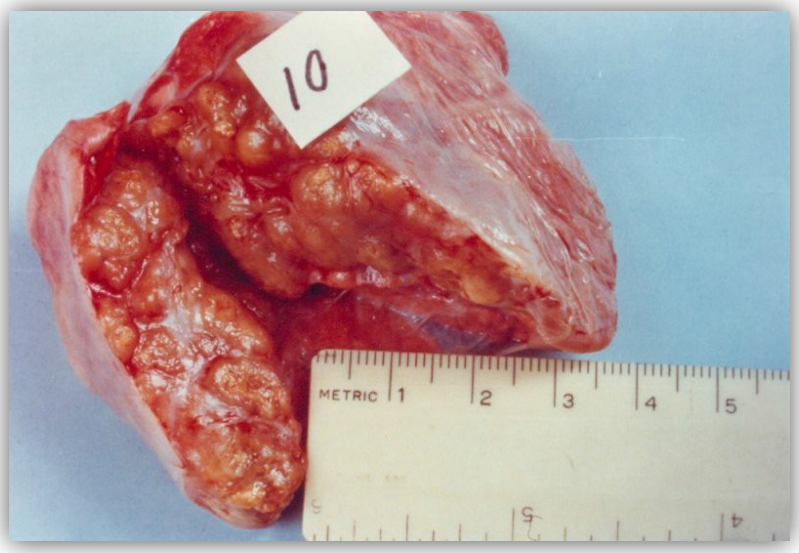
These lesions can be confused with other granulomatous diseases or neoplasms such as actinobacillosis, carcinoma, or mycotic infections.



Lesion 5

Lesion 5 - Lungs, Bronchial and Mediastinal Lymph Nodes

There are focal raised areas in the lung. The incised lesions are composed of tubercles on closer examination. The bronchial lymph node at the center of the photograph and mediastinal lymph node at the lower right are also affected. Again, these lesions can appear similar to lymphoma or granulomatous disease caused by agents other than mycobacteria.



Lesion 6

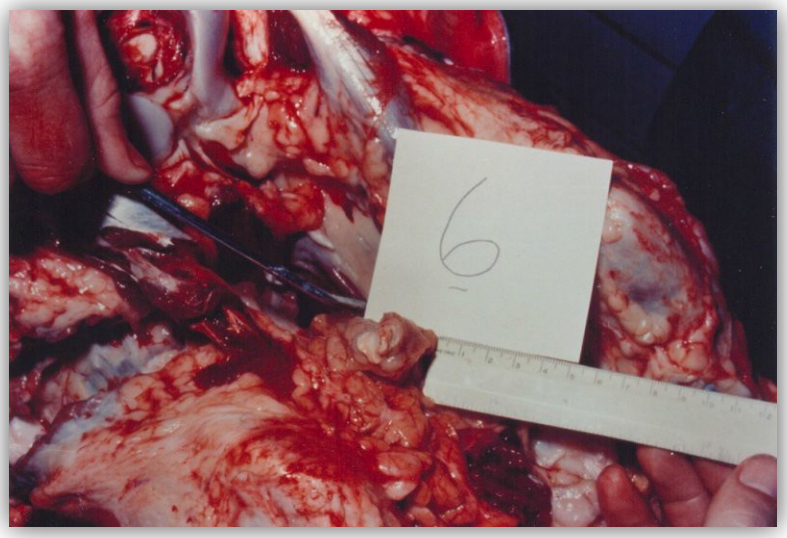
Lesion 6 - Coalescing Tubercles

This image demonstrates tubercles which coalesced and expanded the lymph node. A single enlarged lymph node may be the only evidence of tuberculosis in some animals.

Lesion 7

Lesion 7 - Tubercles on the Ribs

This photo shows tubercles along the pleural surface of the ribs. These tubercles are the body's reaction to mycobacteria that were in the thoracic cavity. The hyperemia and redness of the lesions suggest the infection is active, in contrast with an older lesion which could have a more thickened capsule, necrotic center, and calcification.



Lesion 8

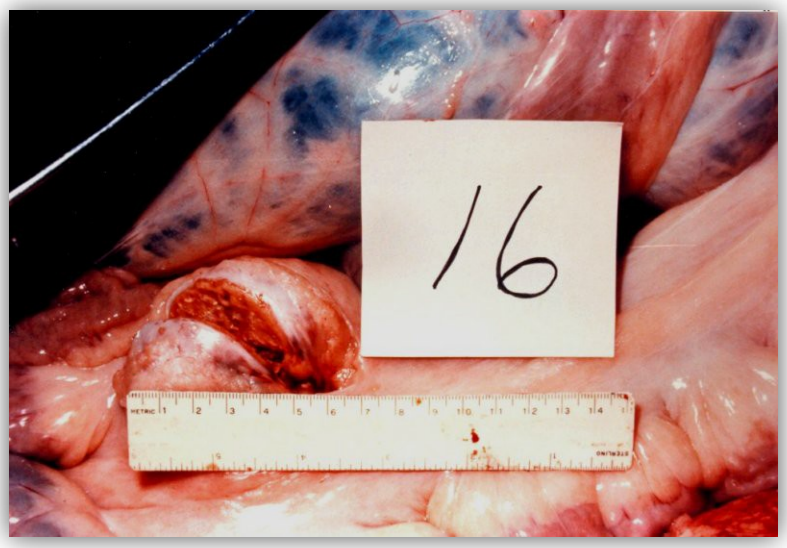
Lesion 8 - Retropharyngeal Lymph Node
Incised lymph node - This tubercle has a liquid center, which is somewhat different than most tubercles which have a mineralized core.

Lesion 9

Lesion 9 - Retropharyngeal Lymph Node
The enlarged node is shown with cut surfaces. The lesions in this enlarged lymph node resemble those that are due to neoplastic masses or infectious agents other than mycobacteria.

Lesion 10

Lesion 10 - Small Yellow Tubercles
This close-up shows that the normal lymph node tissue has been almost completely replaced with granulomatous inflammation.



Lesion 11

Lesion 11 - Mesenteric Lymph Node
Enlarged node with a tuberculous lesion.

Lesion 12

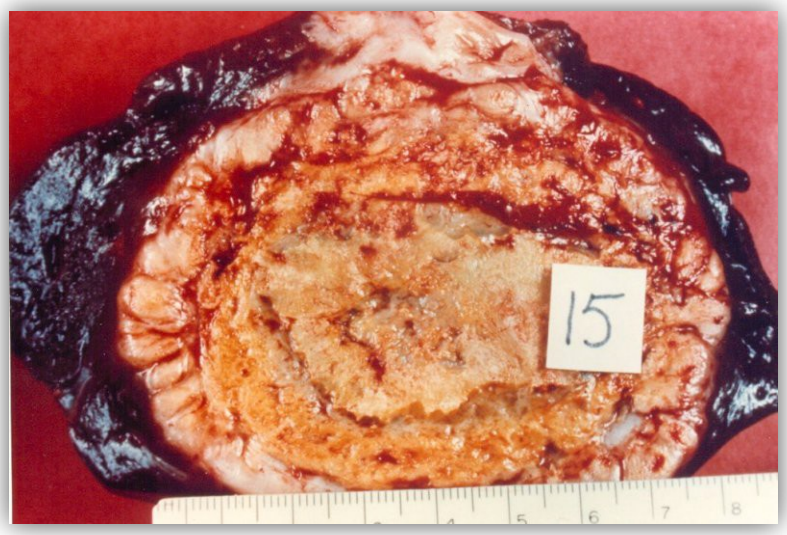
Lesion 12 - Enlarged Mesenteric Lymph Node

Enlarged photograph of the Lesion 11 lymph node showing the granulomatous reaction in cross section - The caseous ("cheesy") necrosis of the lymph node is typical of a granulomatous reaction.

Lesion 13

Lesion 13 - Liver Lesion

Tuberculous granulation necrosis in the liver - The white area on the surface of the liver is the fibrous encapsulation surrounding the lesion.



Lesion 14

Lesion 14 - Enlarged Liver Lesion
The thick capsule can be seen on cross-section. The solid caseous necrosis in this tuberculosis lesion is in contrast to abscesses caused by many types of bacteria which have a liquid necrotic center.

Submission of Tuberculous Lesions or Thoracic Granulomas in Regular Kill Animals

Nonreactors

1. All lesions resembling tuberculosis should be submitted from all regular kill cattle. This includes adults, feeder cattle, and calves. Examples of some types of lesions to submit are depicted in the previous photographs in this manual.

2. Other thoracic granulomas should also be submitted from all classes of cattle except those considered to be caused by coccidioidomycosis found in feedlot steers and heifers. This type of lesion need not be submitted.

3. In swine, submit specimens only from animals having generalized thoracic granulomas.

Identifying Devices and Procedures

Place all identifying devices (backtags, eartags, etc.) in the plastic bag provided in the TB mailer for shipping with the specimen. When removing, or supervising the removal of, identification devices, try to:

1. Leave a small amount (dime sized) of tissue associated with the tag; and

2. Remove back tags using pliers rather than cutting the hair. The DNA needed for tissue matching is located in the root bulbs of the hair, not in the shaft.

If a blood sample is collected for concurrent brucellosis testing, the identifying devices should go with the TB sample submission and a VS Form 1-16 should go with the blood sample. At official establishments where APHIS contractors collect brucellosis blood samples and identification, IPP should ask the brucellosis sample collector for the identification tags for any animal(s) from which granulomas are submitted to the laboratory prior to the end of the shift.

Correct correlation of identification devices with the affected carcass is essential for an accurate traceback to a herd of origin. The following examples are recommended identification procedures provided by FSIS:

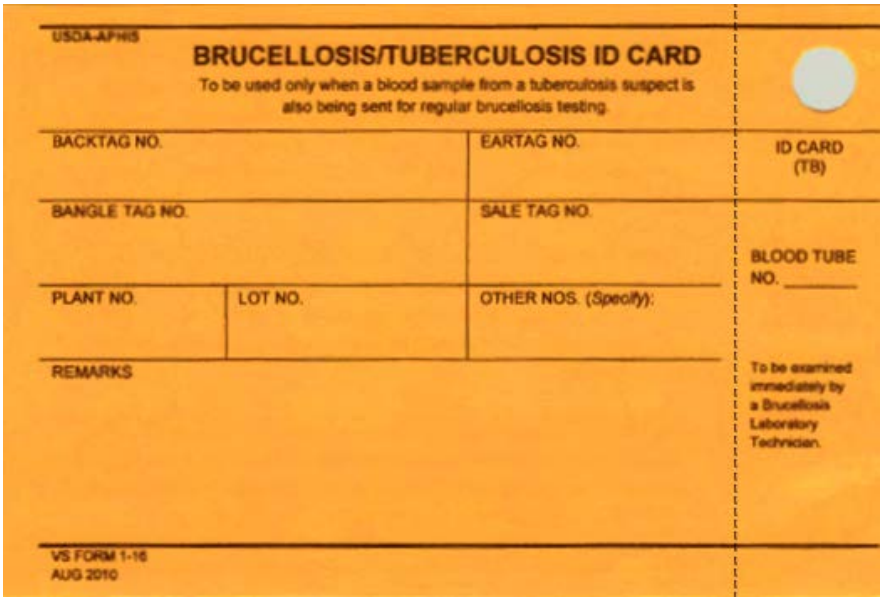
1. A "house tag" should be placed in a plastic bag with the other identification devices, and correlated with the carcass until viscera inspection is completed.

2. In establishments where brucellosis surveillance is being conducted, a "house tag" should be placed in the plastic bag along with the brucellosis blood sample and man-made identification devices. Retain the plastic bag, or alternative, in such a way as to directly relate it to the carcass until postmortem examination and blood sample testing have been completed.

3. When M-branded cattle (steers imported from Mexico) have been identified on the kill floor, plant employees will collect metal ear tags and place them in the plastic bags containing house tags.

   NOTE: A house tag is a sequentially numbered tag provided by the slaughter establishment indicating the order in which the animal was slaughtered during the shift. House tags are typically used to identify and track a particular carcass and corresponding detached head and/or other parts until post mortem inspection is completed. House tags are often used to satisfy requirements in 9 CFR 310.2(a).

VS Form 1-16 - for use when sending concurrent brucellosis samples and TB specimen



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BRUCELLOSIS/TUBERCULOSIS ID CARD

To be used only when a blood sample from a tuberculosis suspect is also being sent for regular brucellosis testing.

BACKTAG NO.		EARTAG NO.	ID CARD (TB)
BANGLE TAG NO.		SALE TAG NO.	BLOOD TUBE NO. _____
PLANT NO.	LOT NO.	OTHER NOS. (*Specify*):	
REMARKS			To be examined immediately by a Brucellosis Laboratory Technician.

VS FORM 1-16
AUG 2010

Record the blood sample number on the small end of the form. Then remove the small end and attach it to the paperwork for the day's brucellosis blood samples.

Record ID numbers on the large end of VS Form 1-16 and place it in the plastic bag with the blood sample being sent to the regular brucellosis laboratory.

Preparation of Specimens

1. Remove excess fat.

2. Divide lesions in half. Place one portion in formalin for histopathology and place the remaining portion in borate for culture.

3. BUFFERED FORMALIN PORTION: Cut specimen, including normal tissue surrounding lesion, into slices approximately 1 cm (½ inch) thick prior to placing in formalin.

4. BORATE PORTION: Place the intact portion of the sample into borate. DO NOT cut the sample into slices.

5. Maximum tissue to preservation ratio is: Formalin - 1:10; Borate - 1:1.

6. If insufficient tissue is available, send sample in formalin only.

7. SBS is a supersaturated solution. Crystals in the bottle are frequently seen and are normal.

8. Reseal lids of both containers with electrical tape.

9. Be sure to write the identification numbers or the retain tag number on the bottle labels.

10. No refrigeration is required but DO NOT FREEZE as freezing damages the specimens.

Reporting Forms

VS Form 6-35, Report of Tuberculosis Lesions or Thoracic Granulomas in Regular Kill Animals

1. This form is used for the submission of specimens from regular kill animals only (not reactor or suspect cattle). The VS Form 10-4 should be used when tissues are submitted from TB reactors or suspects.

2. Complete lines 1-21 of VS Form 6-35. An example of the form is on the following page. Include as much information as is available. Any information is helpful in a traceback. An electronic version of the form can be found at http://www.aphis.usda.gov/library/forms/pdf/VS_Form6_35.pdf.

Note: Be sure to include a telephone number in item 18 if the carcass is being retained pending laboratory results.

See reverse for instructions.

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0579-0146. The time required to complete this information collection is estimated to average .16 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

OMB APPROVED 0579-0146 Exp.: 11/30/2016

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
VETERINARY SERVICES

REPORT OF TUBERCULOSIS LESIONS OR THORACIC GRANULOMAS IN REGULAR KILL ANIMALS

1. TYPE OF INSPECTION ☐ STATE ☐ FEDERAL
2. ESTABLISHMENT NUMBER
3. SPECIES ☐ BOVINE ☐ CERVINE ☐ PORCINE ☐ BISON ☐ OTHER (*Specify*)
4. DATE SLAUGHTERED
5. LOT NUMBER
6. NUMBER IN LOT
7. NUMBER WITH LESIONS
8. ESTABLISHMENT NAME AND ADDRESS (*Include ZIP Code*)
ESTABLISHMENT PHONE NUMBER
ESTABLISHMENT FAX NUMBER
9. NAME AND ADDRESS OF OWNER (*Include ZIP Code*)
10. NAME AND ADDRESS OF SHIPPER (*Include ZIP Code*)
11. NAME AND ADDRESS OF MARKET OR BUYER (*Include ZIP Code*)

12. ANIMAL IDENTIFICATION (*Place all ID devices in box with tissues to National Veterinary Services Laboratories*)

A. OFFICIAL PERMANENT ID/EAR TAG
D. CARCASS NUMBER
B. SALE/BACK TAG NUMBER
E. RETAIN TAG NUMBER
C. OTHER ID (*Brand, Tattoo, Bangle Tag*)
F. AGE
G. SEX
H. BREED/COLOR

13. POST MORTEM REPORT

KEY: / = SLIGHT \ = WELL MARKED X = EXTENSIVE CS = CASEOUS CL = CALCIFIED

HEAD (A)		CERVICAL (B)		BRONCHIAL (C)		MEDIASTINAL (D)		LUNG (E)		PORTAL (F)		MESENTERIC (G)		OTHER (H)
CS	CL	CS	CL	CS	CL	CS	CL	CS	CL	CS	CL	CS	CL	

	WEIGHT	SCALE	ESTIMATED
14. DRESSED			
15. LIVE			

16. COMMENTS

17. CHECK ☐ IF CARCASS RETAINED PENDING LAB RESULTS

18. SEND TEST REPORT TO (*Select One*):
☐ ESTABLISHMENT FAX NUMBER (*BLOCK 8*) ☐ ESTABLISHMENT POSTAL ADDRESS (*BLOCK 8*)
☐ OTHER FAX OR EMAIL (*Specify*) _____

19. NAME OF FOOD INSPECTOR (*Type or Print*)
20. NAME OF VETERINARIAN (*Type or Print*)
21. DATE COMPLETED

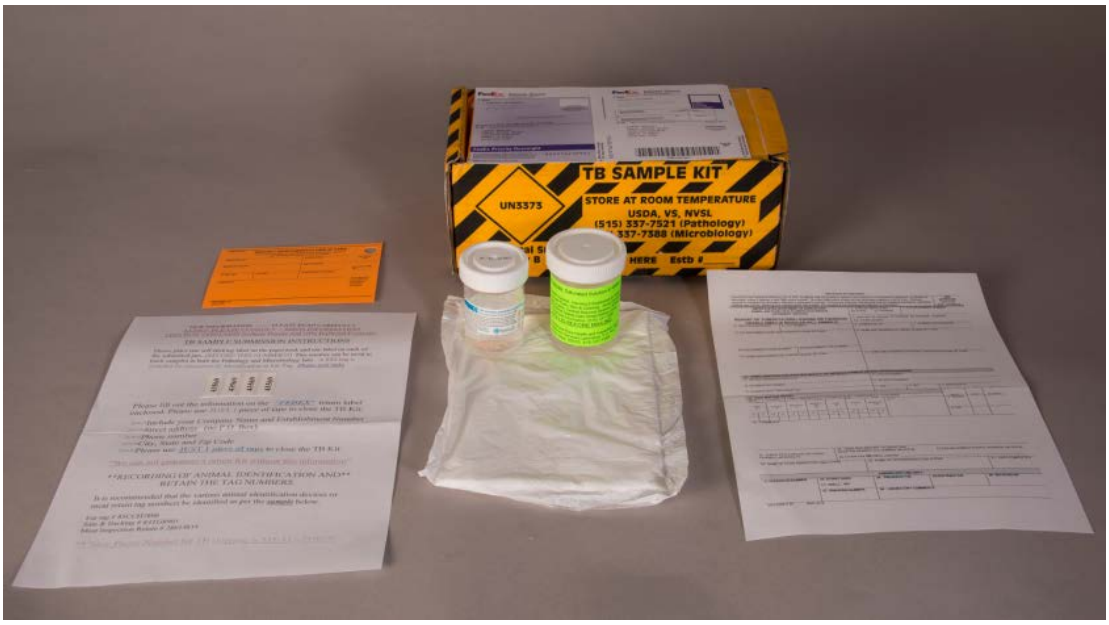
LABORATORY USE ONLY

22.ACCESSION NUMBER
23. ID ENCLOSED ☐ YES ☐ NO
24. PRESERVATIVE
25.DISTRIBUTION
26. RECIEVED BY
27. TRACKING NUMBER
28. LABORATORY COMMENTS

VS FORM 6-35 MAR 2014

Packaging and Shipping TB Specimen

Use the special 2 bottle TB black and yellow mailer, available from the National Veterinary Services Laboratories (NVSL), for each carcass.



In the large re-sealable plastic bag place the:

1. Styrofoam holding the sealed bottles containing the specimen
2. Absorbent pad (on top of specimen)
3. Small re-sealable plastic bag containing ID (on top of absorbent pad)

Place the completed VS Form 6-35 in the black and yellow shipping container, outside of the large re-sealable plastic bag.

Attach the FedEx mailing label with the complete return address and establishment number to the outside of the black and yellow mailer.

Ship the mailer to NVSL at the following address:

USDA APHIS
National Veterinary Services Laboratories
1920 Dayton Avenue
Ames, IA 50010
Telephone number: 515-337-7212

Tuberculosis Glossary

(Terms Often Used in Diagnostic Histopathology of Bovine Tuberculosis)

1. **Acid-fast** - A differential staining characteristic of mycobacteria. Not easily decolorized by acid.

2. **Calcification** - A hardening of tissue due to mineralization of necrotic tissue. Calcification is often seen in bovine tuberculosis lesions.

3. **Capsule** - A fibrous enveloping structure. Tuberculosis granulomas are often enclosed by a connective tissue capsule.

4. **Caseation** - A form of necrosis in which the tissue is changed into a dry, amorphous, "cheesy" mass.

5. **Compatible for Tuberculosis** - Terminology for the diagnosis of bovine tuberculosis in which acid-fast bacteria are seen in a typical tubercular granuloma by microscopic examination.

6. **Granuloma** - Nodular inflammatory lesions, variably sized, firm, chronic masses.

7. **Hematoxylin and Eosin Stain** - The stain routinely used to examine tissue sections microscopically for cellular detail.

8. **Liquefaction** - A form of necrosis in which the necrotic material is a liquid.

9. **Necrosis** - Morphologic changes indicative of cell death.

10. **Tubercle** - The granulomatous lesion of tuberculosis, typically consisting of a caseous center surrounded by epithelioid cells and a connective tissue capsule.