

Tuberculosis Sample Submission Manual for Meat Inspection Personnel

**USDA APHIS Veterinary Services
Ruminant Health Programs Staff
National Center for Animal Health Programs
4700 River Road, Unit 43
Riverdale, Maryland 20737**

Revised November 2005

Table of Contents

Introduction	2	
Lesions of Mycobacterium bovis		
Bronchial Lymph Node	4	
Bronchial Lymph Node	4	
Bronchial Lymph Node	5	
Lungs, Bronchial, and Mediastinal Nodes	6	
Enlarged Node with Tubercles	6	
Tubercles on the ribs	7	
Retropharyngeal Lymph Node	7	
Retropharyngeal Lymph Node	8	
Small Tubercles	8	
Mesenteric Lymph Node	9	
Mesenteric Lymph Node	9	
Liver Lesion	10	
Enlarged Liver Lesion		10
Submission of Tuberculous Lesions or Thoracic Granulomas		
	11	
Identifying Devices and Procedures		
	11	
Preparation of Specimens		
	12	
Reporting Forms		
	12	
VS Form 6-35	12-13	
VS Form 10-23	14	
Tuberculosis ID Card, VS Form 1-16	14	
NVSL Sample Submission Mailer	15	
Tuberculosis Glossary		
	16	

Introduction

The Cooperative State Federal Bovine Tuberculosis Eradication program began in 1917. For many years, the program involved testing of all cattle and slaughter of all test reactors. As a result of this area testing, the prevalence of bovine TB in the U. S. today is at a very low level estimated to be .0009%. Therefore, routine test and slaughter practices are an extremely inefficient way of detecting new cases of the disease.

Today, emphasis is placed on detecting bovine tuberculosis during regular kill procedures at slaughter. As the number of cattle routinely tuberculin tested has decreased, the importance of slaughter surveillance has significantly increased. In recent years, almost 95% of the TB infected herds have been detected through slaughter traceback and subsequent epidemiological investigations.

The information provided to APHIS through slaughter submissions allows the APHIS VMO to effectively investigate herds that are thought to be infected with bovine tuberculosis. The information provided by meat inspection personnel is the first and most important step in this investigation process. Accurate tissue selection and carcass identification leads to the prompt identification of infected herds.

The success of the TB eradication program depends to a large degree on the efforts of the State and Federal meat inspectors. Submitting lesions resembling tuberculosis to the National Veterinary Services laboratory (NVSL) in Ames, Iowa is important for the following reasons:

- 1) To find newly infected animals and herds, a diagnosis of tuberculosis must first be made. Obviously, a diagnosis can only be made if a sample is submitted.
- 2) As the level of TB further decreases, it becomes important to have an adequate number of granulomatous lesions submitted to effectively detect a tuberculous herd.
- 3) Since many granulomas are identical in appearance, it is imperative that all granulomas be submitted for evaluation.

The photographs on the following pages are presented as an aid for 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 as close ups in the inspector's office when preparing lesions for submission.

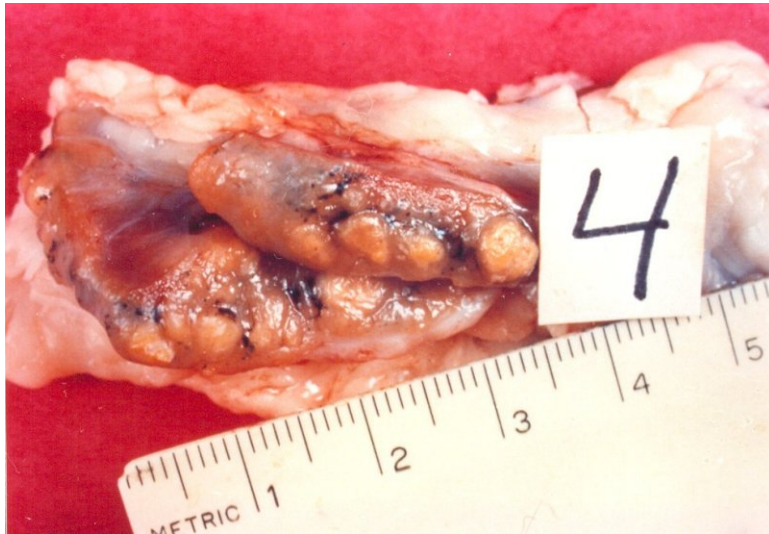
All photographed lesions were confirmed as being caused by *M. bovis*. Lesions were obtained from several animals during the evaluation of a herd where 45 reactor animals had the following distribution of tuberculous lesions:

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 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. Most TB lesions in recent years have been found in feeder cattle.

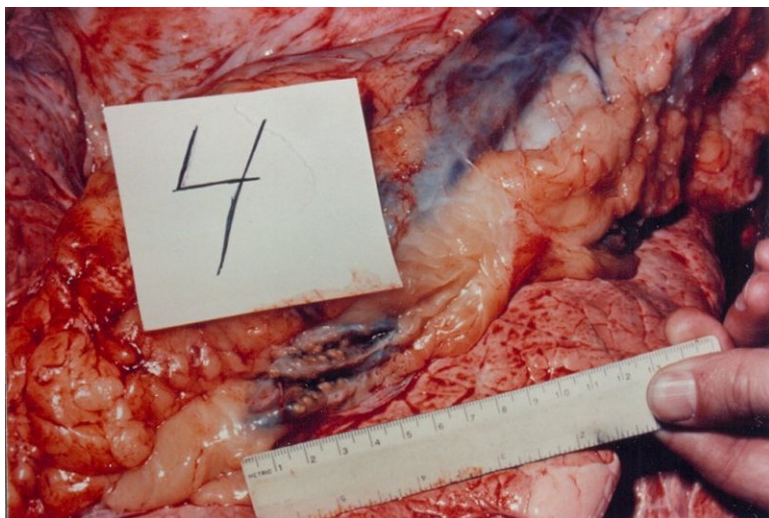
This manual is prepared by APHIS as a guide for identifying and preparing tissue samples for submission to NVSL. The first section gives pictorial examples of common tuberculous lesions, while the remainder of the manual is intended as a handbook for the proper submission of suspect granulomatous lesions. 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



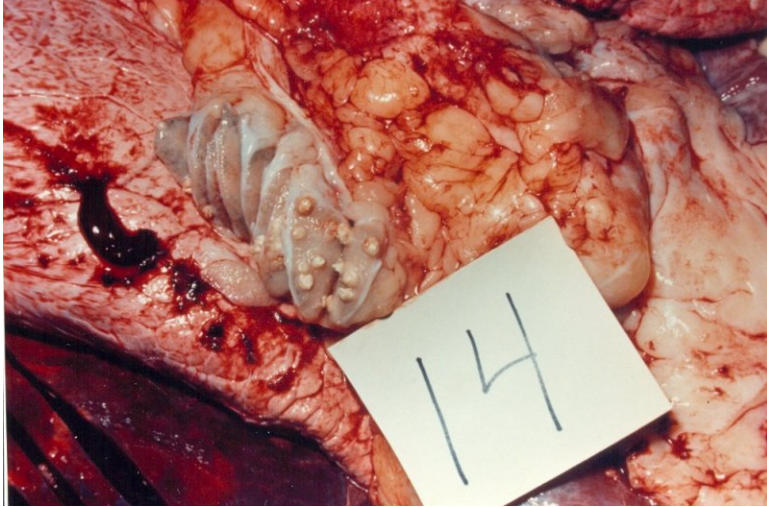
Lesion #1 (sample 4 to left):

Incised bronchial lymph node with tubercles (light colored, small rounded firm nodules). These 1 millimeter to 20 millimeter lesions are those typically seen when nothing is obscuring the lesions, the ideal situation.



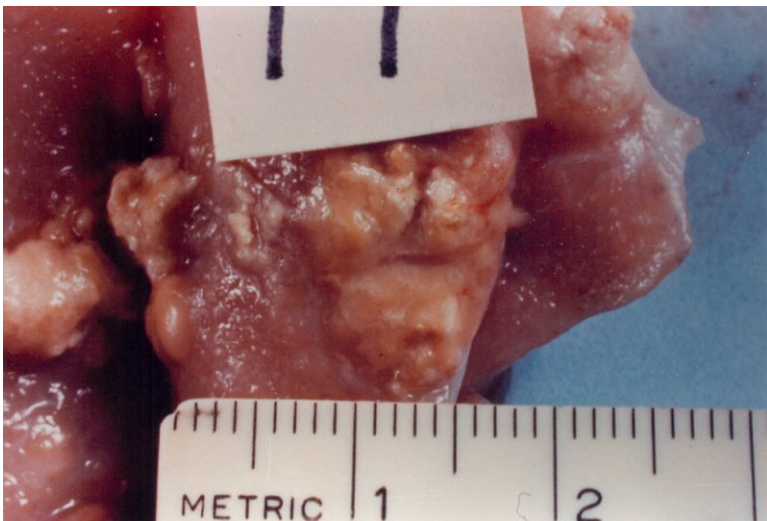
Lesion #2 (sample 4 to right):

Tubercles in a bronchial lymph node. The discreet lesions are the typical yellowish color seen when not hidden by accompanying infection. Some of the adjacent tubercles have started to grow together.



Lesion #3 (sample 14 to left):

Incised bronchial lymph node with several discrete tubercles. These look similar to abscesses.



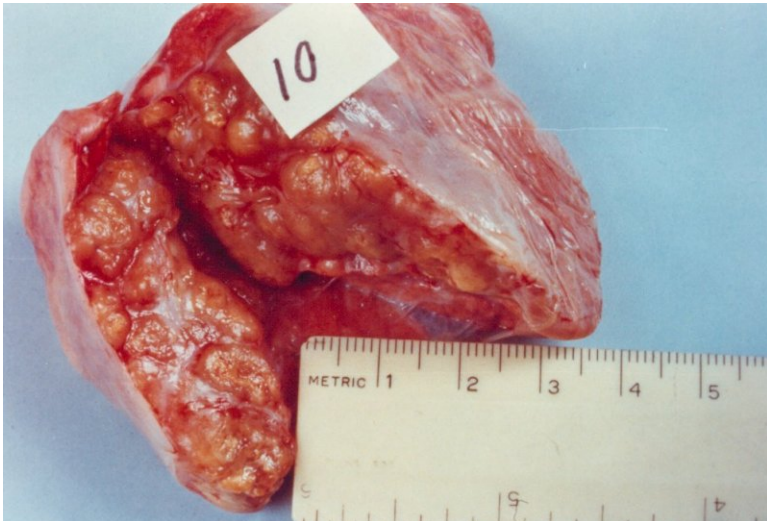
Lesion #4 (sample to left):

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



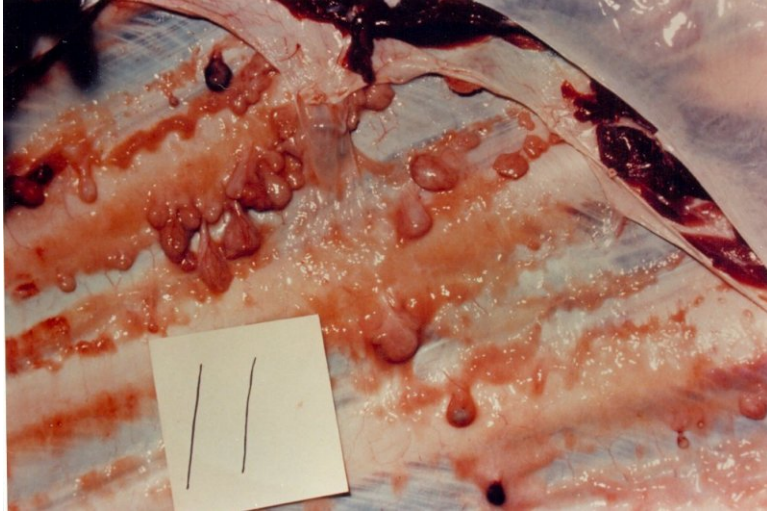
Lesion #5 (sample to right):

This photo shows bulged areas in the lungs. The incised lesions show 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 could suggest a lymphoma or other granulomatous disease.



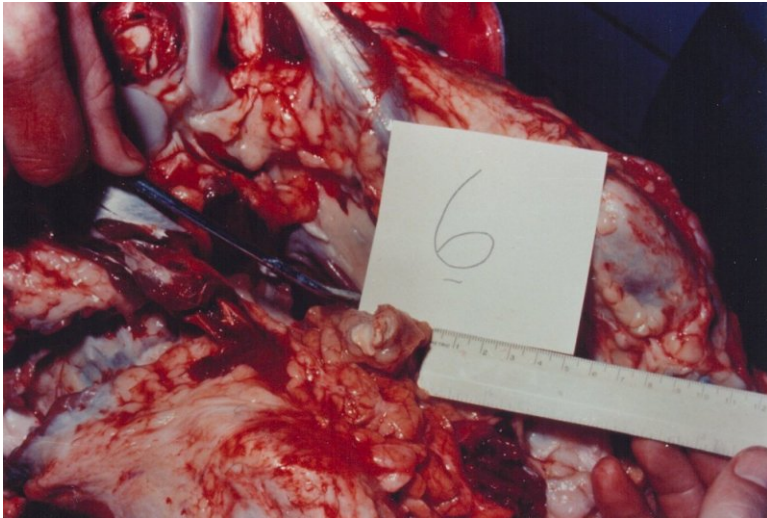
Lesion #6 (sample 10 to left):

This magnification shows tubercles coalescing in the enlarged lymph node. A single lymph node like this may be enlarged with no other evidence of disease or spread.



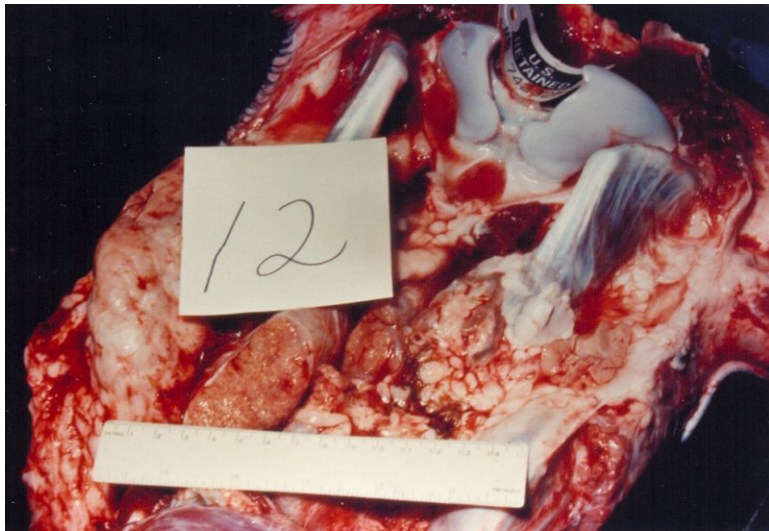
Lesion #7 (sample 11 to left)

This photo shows tubercles along 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 and acute in this case, in contrast with an older lesion which could have a thickened capsule, necrotic center, and/or calcification.



Lesion #8 (sample 6 to right):

Retropharyngeal lymph node with incised tubercle. This tubercle has a moist appearance because of a greater amount of purulence than is seen in the "typical" TB lesion. The tubercle resembles an abscess because of a secondary bacterial infection.



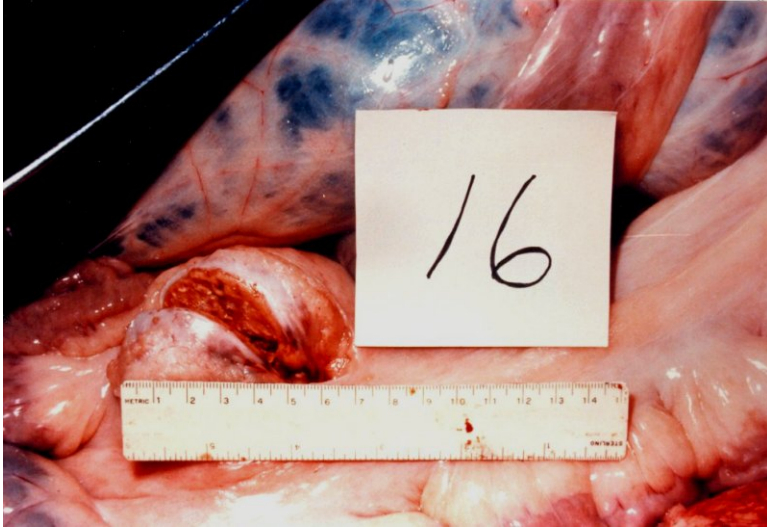
Lesion #9 (sample 12 to left):

Enlarged retropharyngeal lymph node shown with cut surfaces. This enlarged lymph node can also suggest a neoplasm or other infectious process.



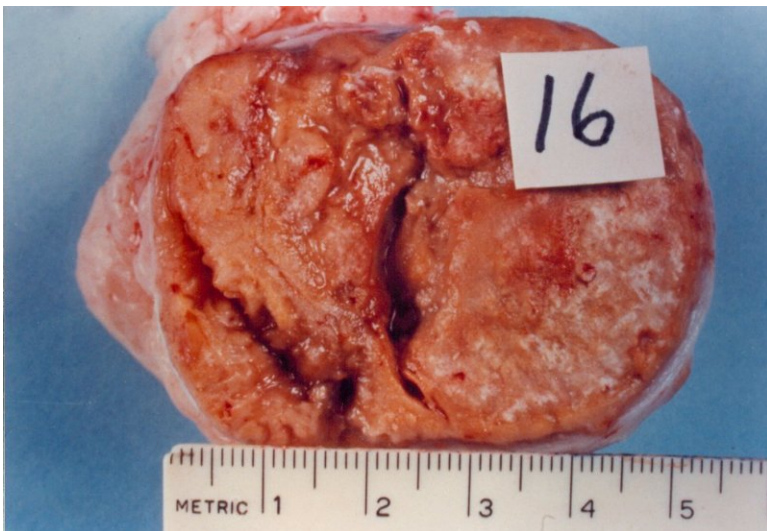
Lesion #10 (sample 12 to the left):

This close-up shows very small yellow tubercles that may not be easily observed in the usual inspection procedure.



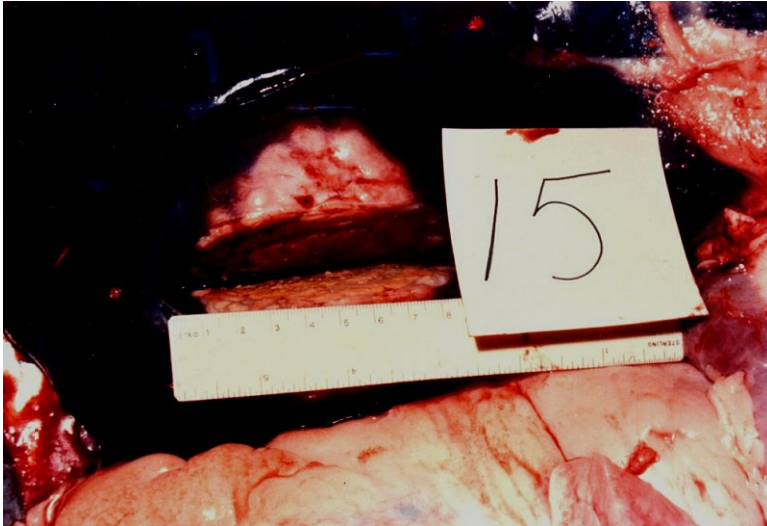
Lesion #11 (sample to right):

Enlarged mesenteric lymph node with a tuberculous lesion.



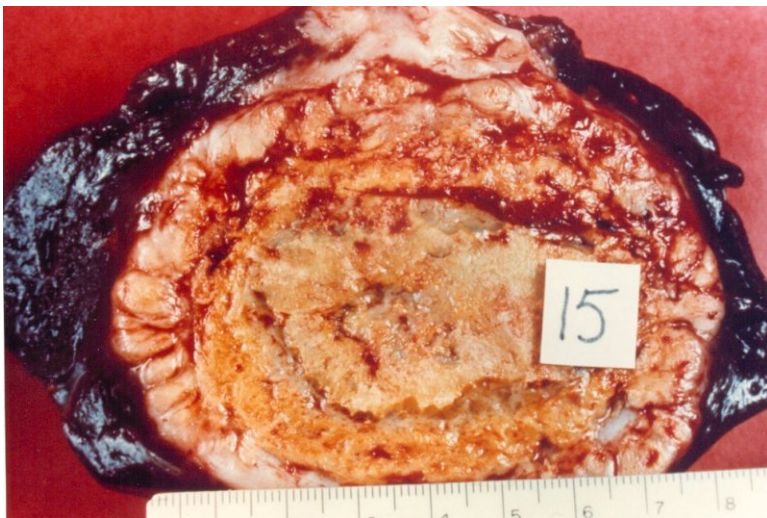
Lesion #12 (sample 16 to left):

Enlarged photograph of the above 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 (sample 15 to left):

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



Lesion #14 (sample 15 to right):

On cross-section, the thick capsule can be seen. The solid caseous necrosis contrasts with that seen in an abscess.

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, ear tags, etc.) in a plastic bag in the box with the specimen. Do not remove the sponge (see photo). If a blood sample has been collected from this animal, the identifying devices still go with the TB sample Submission and a VS Form 1-16 should go with the blood sample.

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:

- A. When nonmature cattle (steers, heifers, and calves) are slaughtered, a "house tag" should be placed in a plastic bag with the other identification devices, attached to the carcass, and remain so attached until viscera inspection is completed.
- B. When mature cattle (cows and bulls) are slaughtered, a "house tag" should be placed in the plastic bag along with the brucellosis blood sample and man-made identification devices.
- C. 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.

Preparation of Specimens

1. Remove excess fat.
2. Divide lesion, including the normal tissue, into blocks approximately 1/4" - 1/2" thick.
3. Place 1/2 of the tissue in buffered formalin for histopathology. Place the remainder of the tissue in Sodium Borate Solution (SBS) for bacteriologic examination.
4. Reseal lids of both containers with electrical tape.
5. Be sure to write the identification numbers or the retain tag number on the bottle labels.

Other Notes

1. Maximum tissue to preservative ratio: formalin 1:10 SBS 1:1
2. If insufficient tissue is available, send sample in formalin only.
3. SBS is a supersaturated solution. Crystals in the bottle are frequently seen and is normal.

Reporting Forms

1. VS Form 6-35

This form is used for the submission of specimens from regular kill animals only (not reactor or suspect cattle). The original and one copy go with the specimen and the third copy is retained by the inspector. The VS Form 10-4 should be used when tissues are submitted from TB reactors or suspects.

A. Complete lines 1-20 of VS Form 6-35 as shown in the example on the following page. include as much information as is available. Any information is helpful in a traceback.

USDA - APHIS - VETERINARY SERVICES		1. TYPE INSPECTION <input type="checkbox"/> STATE <input type="checkbox"/> FEDERAL		2. ESTABLISHMENT NO.													
REPORT OF TUBERCULOSIS LESIONS OR THORACIC GRANULOMAS IN REGULAR KILL ANIMALS		3. SPECIES: <input type="checkbox"/> BOVINE <input type="checkbox"/> CERVINE <input type="checkbox"/> PORCINE <input type="checkbox"/> BISON <input type="checkbox"/> OTHER (Specify)															
4. DATE SLAUGHTERED		5. LOT NO.	6. NO IN LOT	7. NO WITH LESIONS													
8. ESTABLISHMENT NAME & ADDRESS (Include Zip Code)			9. NAME & ADDRESS OF OWNER (Include Zip Code)														
10. NAME & ADDRESS OF SHIPPER (Include Zip Code)			11. NAME & ADDRESS OF MARKET OR BUYER (Include Zip Code)														
12. ANIMAL IDENTIFICATION (Place all ID devices in box with tissues to NVSL)																	
A. EAR TAG/OTHER OFFICIAL PERMANENT ID #			D. CARCASS #														
B. SALE/BACK TAG #		E. AGE	SEX	BREED/COLOR													
C. OTHER ID (brand, tattoo, bangle tag)			F. RETAIN TAG #														
13. POST MORTEM REPORT																	
KEY <input checked="" type="checkbox"/> SLIGHT <input type="checkbox"/> WELL MARKED <input checked="" type="checkbox"/> EXTENSIVE CS - CASEOUS CL - CALCIFIED				WEIGHT	SCALE	ESTIMATED											
HEAD (A)	CERVICAL (B)		BRONCHIAL (C)		MEDIASTINAL (D)		LUNG (E)		PORTAL (F)		MESENTERIC (G)		OTHER (H)		14. DRESSED		
CS	CL	CS	CL	CS	CL	CS	CL	CS	CL	CS	CL	CS	CL		15. LIVE		
16. COMMENTS																	
17. CHECK <input type="checkbox"/> IF CARCASS RETAINED						18. FAX NO. (Include area code)			18a. TELEPHONE NO. (Include area code)			19. RESERVED					
PENDING LAB RESULTS (SEE * ON BACK)																	
20. NAME OF FOOD INSPECTOR (Type or Print)									21. NAME OF VETERINARIAN (Type or print)						22. DATE COMPLETED		
LABORATORY USE ONLY																	
23. DATE RECEIVED			24. ID ENCLOSED ("X" one) <input type="checkbox"/> YES <input type="checkbox"/> NO			25. PRESERVATIVE			26. DISTRIBUTION			27. RECEIVED BY					
28. ACCESSION #			29. CASE #			30. TRACKING # A - 444602			31. SAMPLE #								

Note: Be sure to include a telephone number in item 17 if the carcass is being retained pending laboratory results. Also attach a VS Form 10-23 (orange RETAINED) to the mailing box if the

carcass is retained. Histopathology results usually take about a week but bacteriology results require up to 8 weeks and will be included in the final report.

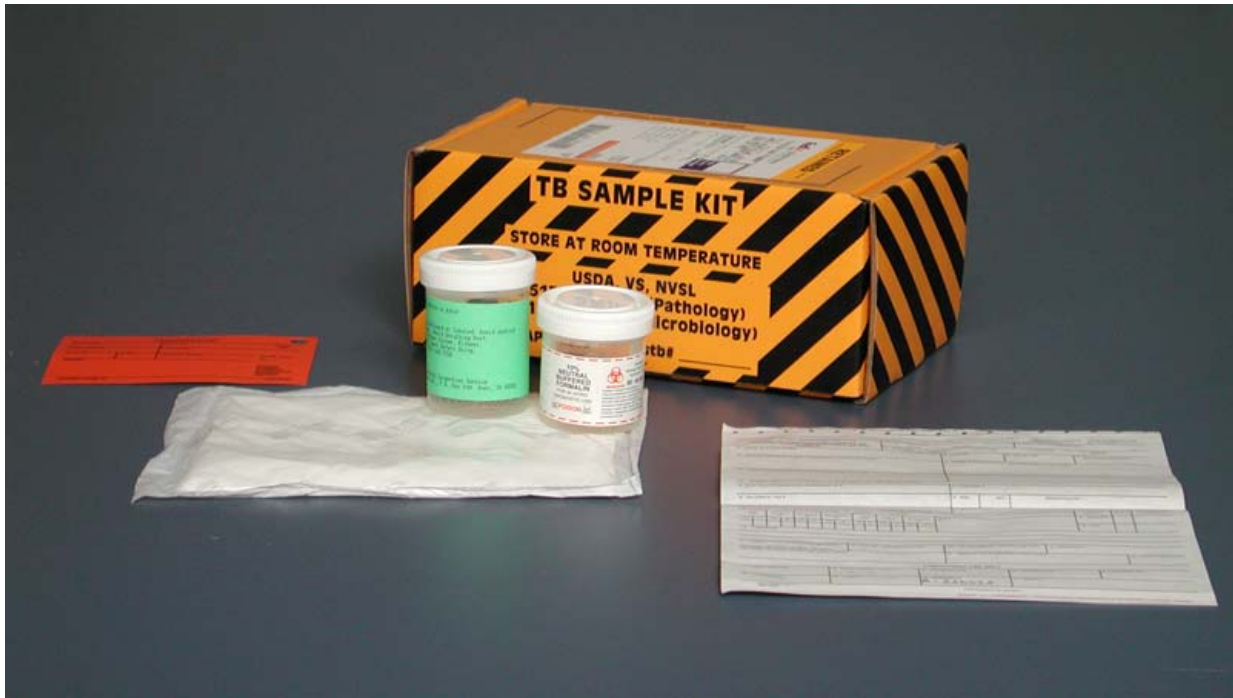
VS Form 1-16

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

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 blood samples.

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

Use the special black and yellow striped box with the two bottles inside (formalin and SBS).



Ship this container to NVSL at the following address:

NVSL
1800 Dayton Road
Ames, IA 50010

If the carcass is being retained pending laboratory results, be sure to attach the orange RETAINED sticker (VS Form 10-23) to the outside of the container.

Include the completed VS Form 6-35 in the shipping container, between the polystyrene box and the outside mailer.



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. **Auramine-0 Stain** - A fluorochrome stain that utilizes auramine-0 to detect acid fast bacteria with a fluorescent microscope.
3. **Calcification** - A hardening of tissue due to the deposition of calcium solids. Calcification is often seen in old tubercular granulomas.
4. **Capsule** - An enveloping structure. Tuberculosis granulomas are often enclosed by a connective tissue capsule.
5. **Caseation** - A form of necrosis in which the tissue is changed into a dry, amorphous, "cheesy" mass.
6. **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.
7. **Granuloma** - Nodular inflammatory lesions, usually small or granular, firm, persistent, and containing compactly grouped mononuclear phagocytes.
8. **Hematoxylin and Eosin Stain** - The stain routinely used to examine tissue sections microscopically for cellular detail.
9. **Langhans's Giant Cell** - A multi-nucleate giant cell often found in a tubercle.
10. **Liquefaction** - A degree of necrosis that converts tissue into a liquid form.
11. **Lymphocyte** - A small mononuclear circulatory white blood cell formed in lymphoid tissue throughout the body.
12. **Macrophage** - Large mononuclear circulatory phagocytes.
13. **Micron** - 1/1,000 of a millimeter.
14. **Mycolic Acid** - An organic compound found in mycobacteria which is believed to be responsible for the acid-fast characteristic.
15. **Necrosis** - Death of tissue.
16. **Neutrophil** - A polymorphonuclear leucocyte.

17. **Phagocytosis** - The process of ingestion and digestion by cells of solid substances, such as other cells, bacteria, or foreign particles.

18. **Reticulo-endothelial Cell** - A phagocytic, mononuclear cell having both endothelial and reticular attributes.

19. **6-35** - VS Form 6-35, The form used for the submission of tuberculosis granulomas to NVSL for histopathologic and bacteriologic examination.

20. **Suggestive of Tuberculosis** - Terminology for the diagnosis of bovine tuberculosis in which a typical tubercular granuloma is seen microscopically, but no acid-fast bacteria can be found.

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