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United States  
Department of  
Agriculture

VETERINARY SERVICES MEMORANDUM NO. 552.15

Animal and Plant  
Health Inspection  
Service

SUBJECT: Instructions and Recommended Procedures for Conducting Tuberculosis Tests in Cattle and Bison

Veterinary Services

TO: VS Management Team (VSMT)  
Directors, VS

Washington, DC  
20250

I. PURPOSE

The purpose of this memorandum is to provide general guidelines for conducting tuberculosis (TB) tests in cattle and bison under the national cooperative State-Federal bovine TB eradication program. These guidelines are in accordance with the Bovine Tuberculosis Uniform Methods and Rules (UM&R).

II. CANCELLATION

This memorandum replaces Veterinary Services (VS) Memorandum 552.15, dated September 28, 1982, which is hereby canceled.

III. GENERAL

The primary objective of the TB eradication program is to aid in the detection and removal of all cattle and bison infected with or exposed to *Mycobacterium bovis* (*M. bovis*). This memorandum presents: (1) field guidelines for using and interpreting diagnostic tests for TB, (2) testing procedures for TB investigations involving high risk herds of cattle and bison, and (3) recommendations for testing animals other than cattle and bison.

IV. PUBLIC HEALTH CONSIDERATIONS

Since bovine TB is a human health hazard, the Area Veterinarian in Charge (AVIC) or the cooperating State official should promptly notify public health officials of the existence of bovine TB in a herd. In addition, the AVIC must maintain liaison with public health officials to ensure VS is notified of human cases within the State.

Owners of *M. bovis* infected or exposed cattle and bison must be advised of the possible danger these animals present to themselves, their families, their employees, and to the general public. The danger of direct contact with infected animals or the consumption of their raw milk and milk products must be thoroughly explained. All persons with possible exposure should be advised to seek medical attention.



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All milk from affected herds must be pasteurized prior to human or animal consumption. Milk from TB reactors must not be used for human or animal consumption. Owners of reactors must be advised to segregate tuberculin reactors from non-reactors and dispose of their milk in a safe manner. Feeding of concentrates to lactating reactors should be stopped or reduced. Milking frequency should also be reduced or discontinued when possible.

TB reactors must be moved in compliance with VS regulations (Title 9 of the *Code of Federal Regulations* (CFR) section [77.17](#)) and their carcasses disposed of in accordance with U.S. Department of Agriculture (USDA), Food Safety Inspection Service (FSIS) regulations (9 CFR [311.2](#)). Carcasses from reactors are passed for cooking or condemned depending on postmortem results, and are never passed without restriction. Exposed animals or animals classified as TB test suspects are handled according to their postmortem findings and may pass without restrictions if evidence of TB is not detected.

Regulatory veterinarians and technicians conducting tests or performing postmortem examinations in affected herds of cattle and bison should be tested at least biennially for TB. The precautions employed when performing postmortem examinations on animals possibly affected with TB are left to the discretion of the examining veterinarian, but should include the use of protective gloves and glasses, mask or respirator, and additional outer clothing.

## V. TUBERCULINS

The following purified protein derivative (PPD) tuberculins are currently used:

1. USDA Bovine PPD Tuberculin (PPD Bovine) - PPD standardized at 1.0 mg protein/ml.
2. USDA Avian PPD Tuberculin (PPD Avian) - PPD standardized at 1.0 mg protein/ml.
3. USDA Bovine Cervical Test PPD Tuberculin (PPD Bovine, Cervical) - PPD standardized at 2.0 mg protein/ml.
4. Balanced PPD Tuberculins - PPD Avian and PPD Bovine tuberculins that have had their protein concentrations adjusted to equalize their biological activity. They are adjusted to give equal responses, on the average, in cattle infected with the same strain.

## VI. INSTRUCTIONS

Only State, Federal, or accredited veterinarians (category 2 under the new veterinarian accreditation system) may conduct TB testing. Accredited veterinarians are only approved to conduct the caudal fold test (CFT). Technicians employed by State or Federal governments and approved by such governments may conduct routine screening TB CFT when directly supervised by State or Federal animal health veterinarians.

Approved State or Federal veterinarians must be notified if a cervical tuberculin test (CT), comparative-cervical test (CCT) or interferon gamma assay needs to be performed. All tuberculin tests conducted for any purpose on cattle and bison are official tests and must be reported to the cooperative State-Federal program officials. Failure to report tuberculin test is sufficient grounds for the removal of an accredited veterinarian status as provided in 9 CFR [161](#).

Inoculation of test animals with vaccines or the administration of drugs, pharmaceuticals, or anthelmintics in conjunction with tuberculin injections should be avoided. Live vaccines and corticosteroid drugs, in particular, may either depress or suppress an animal's immune system and thus reduce its ability to respond to the tuberculin test. Such products should be administered at the time of observation rather than at the time of injection.

### A. Equipment

1. Tuberculin – A VS-approved PPD Bovis tuberculin or specifically approved PPD for the test conducted. All tuberculin must be stored in the dark at 39°F – 46°F and not used after the expiration date. Tuberculin must not be allowed to freeze. Tuberculin that has been frozen should be discarded and not used to perform testing. (See VS Memorandum [552.11](#) for instructions on the handling of tuberculin).
2. Syringe – Disposable 1.0 cc and/or 0.5 cc plastic or glass tuberculin syringes.
3. Needles – 26 gauge, 1 centimeter (3/8 inch) long (the only permitted size).
4. Absorbent cotton or paper toweling.
5. Isopropyl alcohol (70 percent).
6. Lariat, nose tongs, halter, chute, head gate, and/or other equipment needed for adequate restraint.
7. Official identification devices and applicators.

8. Required forms – VS Form 6-22 “Tuberculin Test Record,” or State equivalent and VS Forms 6-22C, “Tuberculin Test Record (Special)” and VS Form 6-22D, “Comparative Cervical Tuberculin Test Results.” VS Form 6-22B, “Tuberculin Test Record Continuation Sheet” is optional for use in large herds.
  9. Electric - preferably cordless - hair clipper with a Number 40 size blade (or equivalent) for clipping injection sites in the cervical region.
  10. Dermal thickness gauge (calipers) for the CCT. The calipers must be approved by the National Center for Animal Health Programs (NCAHP) for use in Ruminant Health Programs.
  11. Heparinized (green top) blood tubes for the gamma interferon test.
  12. Other equipment, as deemed necessary, to ensure safe and efficient completion of testing (e.g., flashlight, icepacks for shipping blood samples, protective equipment, bucket, brush, disinfectant, rubber boots, soap).
- B. Identification
1. All cattle and bison tested shall be individually identified by official ear tags. Such identification must be recorded in its entirety on the test record at the time of injection and should be confirmed at the time of observation.
  2. Additional identification (such as bangle tags, non-official metal ear tags, neck chain numbers, tags, brands, horn numbers, and names) should also be recorded on the test record as supplemental information, but must never be used as the sole method of identification.
  3. When cattle and bison have been tagged with more than one official ear tag, all ear tag numbers must be recorded in their entirety.
  4. The breed, sex, and approximate age in years (not C=Calf or A=Adult) of each animal tested must be recorded on the test record in their entirety.
  5. The owner should be informed of the number of animals injected, and advised to restrict them to the premises until the test is completed in 72 ( $\pm$  6) hours.

### C. Restraint

1. All cattle and bison tested must be sufficiently restrained to permit careful application of the tuberculin injection(s), correct reading of animal identification, careful observation and palpation of the injection sites, proper pre-and post-injection skin fold measurements, and adequate clipping of the cervical area when required. No test should be applied or observed without having the animal restrained in a satisfactory manner.
2. Nose tongs used for restraint must be disinfected between animals.

### D. Injection Techniques

1. Ensure the injection site is free of manure, debris, and hair. If necessary, clean the site to make a sanitary injection. Use soapy water or alcohol with cotton, paper, fiber toweling, or other suitable material. Clip hair for the CT and CCT in cattle and bison.
2. Check the syringe and needle for cleanliness, leakage, proper needle gauge and length of needle.
3. Use a new needle for each animal in order to minimize transmission, or the appearance of transmission, of blood-borne, infectious agents between animals. Exceptions must be approved in writing by the AVIC.
4. Ensure that the injection of tuberculin is intradermal. Insert the needle to its full length between the superficial layers of the skin, withdraw slightly, and inject the tuberculin. If a proper injection is made, a small bleb will appear at the injection site.
5. If a mistake is made during injection (such as not getting the injection intradermal), then steps 1 through 4 should be completed on the opposite side. In addition, the side with the correct injection should be marked - preferably with black marker - and a note should be made on the test chart identifying the mistake and the side used for the correct injection.

### E. Reading the Test

1. Observation and palpation must occur at 72 ( $\pm$  6) hours post-injection for all cattle and bison tested. The injection site for all tuberculin tests in all animals must be observed and palpated. Observation without palpation is not acceptable, and is grounds for suspension of veterinary accreditation under 9 CFR [161](#).

2. The veterinarian who makes the tuberculin injection must be the one who “reads” (determines) the results of that test. Exceptions must be approved in writing by the AVIC.
3. Check identification at the time of reading to verify that every animal injected was presented for observation.
4. All increases in size or sensitivity by the animal will be interpreted as a positive test.
5. All responses to the presumptive tests shall be recorded and the animal classified as suspect unless the reactor classification is indicated.

F. Recording and Reporting Results

1. All responses must be reported on an official test record according to instructions for specific tests.
2. Copies of the test record will be distributed by the testing veterinarian in accordance with cooperative State-Federal policy.

G. Test History Records

1. TB test records must be completed and sent to the State Animal Health or Federal VS office and filed to establish TB test histories on individual herds. It is important that these files provide a source for the test history of each herd tested and of each animal in a herd. Files on TB infected herds and herds investigated for possible TB infection (i.e., VS Form 6-35, 6-4a, 6-4b investigations) must not be destroyed for at least 25 years.
2. TB test histories must be available in State or Federal Animal Health offices in order to:
  - a. Aid the testing veterinarian in interpreting future tuberculin tests. Testing veterinarians should have copies of previous herd test records that may show responding animals and other pertinent test history that they can review before and during a current TB test;
  - b. Aid in tracing the origin of reactors and movements of exposed animals, and in confirming that animals were in the herd on a previous test;
  - c. Evaluate the level of nonspecific sensitivity to a test in a given area; and

- d. Evaluate the response rates on tests as reported by individual testing veterinarians.
3. Because TB test histories are needed for such a variety of reasons, it is imperative for the records to be legible, accurate, complete, and uniform. It is the joint responsibility of the State and Federal officials in charge to see that record systems are maintained in a way that meets all reasonable program needs.

## VII. APPROVED TB TESTS – SPECIFIC INSTRUCTIONS

There are three official TB tests for cattle and bison: CFT, CT, and CCT. In addition, the Interferon Gamma Assay - Bovigam TM is an official test for cattle only. No other tests are official or approved. No other tuberculin shall be applied concurrently with an official test.

### A. CFT

1. Definition – A CFT is the intradermal injection of 0.1 ml PPD Bovine Tuberculin in the caudal fold of the bovine with observation and palpation at  $72 \pm 6$  hours.
2. Use – The CFT is the official presumptive test for cattle and bison except in animals known to have been exposed to *M. bovis*. [Exposed cattle and bison acquired from an affected herd must be tested initially with the CT. The CFT is used on known exposed animals in affected herds only when the use of the CT has not been approved by the AVIC or the State Veterinarian and with the approval of the Designated Tuberculosis Epidemiologist (DTE) and Regional Tuberculosis Epidemiologist (RTE).]
3. Application – This test must be applied by State, Federal, or accredited veterinarians. Under certain conditions State or federally employed animal health technicians may apply the test (see UM&R). Accredited veterinarians may not apply the CFT in affected herds.



Figure 1. This picture illustrates the proper location for intradermal injection of tuberculin for the CFT.

The injection site is in the caudal fold which is a flap of hairless skin that extends from both sides of the base of the tail (proximally) to about 14 centimeters (5 inches) from the base of the tail (distally). The injection site is about 7 centimeters (2 and ½ inches) distal to the base of the tail, well away from the hairline, in the center of the fold (see Figure 1). The testing veterinarian should use the same side for an entire lot or herd tested. Any abnormalities found near the injection site should be noted on the test record so that such abnormalities will not be mistaken for tuberculin responses during test observation. If necessary, the opposite caudal fold may be used. Record on the test chart left or right for the caudal-fold side injected. Collecting blood from the coccygeal vein is not recommended at the time of caudal fold injection. If a blood specimen is desired, collect it after the observation, or use a different site to collect blood.



4. Reading the test – The tail must be raised sufficiently to stretch the caudal fold slightly. Palpate the length of the caudal fold that was injected for responses. Any increase in caudal fold thickness either observed or palpated at the site of injection is considered a response.
5. Recording responses – Record as a response on the official test record any increase in caudal fold thickness at the site of injection. Any increase in caudal fold thickness or other inflammatory response at the site of injection is recorded with a plus (+) sign in the appropriate column of the official test record.
6. Interpretation of the test – All responses to tuberculin injections shall be recorded by a plus (+) sign in the size column of the VS Form 6-22 as indicated above.

The testing official must immediately report such responses to the State Veterinarian or the AVIC of the State where the animal is located so arrangements can be made for a specifically trained veterinarian to test animals that respond to the CFT with an approved supplemental test. The second column under “Results” labeled NRS should be marked with an N (negative) when no increase in caudal fold thickness was detected, with an S (suspect) when a (+) in the size column indicates that increased thickness did occur.

Animals that are suspects on the CFT must be retested with a supplemental test to determine their final classification. Nevertheless, an animal with a response must be classified as suspect except that veterinarians employed by the State or Federal government can classify a CFT responding animals as reactors when in their professional opinion that classification is indicated. The reactor classification would be indicated when the animal or the herd has previously associated with other herds or animals affected with *M. bovis*, when the herd is the likely source of a 6-35 investigation, and in similar high-risk situations.

## B. CT

1. Definition – A CT is the intradermal injection of 0.1 ml of PPD Bovine Cervical Tuberculin in the mid-cervical region of the bovine with observation, palpation, and postinjection measurements at  $72 \pm 6$  hours. The CT is limited for use only in cattle known to have been exposed to *M. bovis*.
2. Use – The CT is used on cattle or bison known to have been exposed to *M. bovis*, and it is the only test approved for use on exposed animals

acquired from an affected herd (VS Form 6-4b investigations). The test is also recommended for testing herds known to be affected with *M. bovis* when approved by the AVIC and the State Veterinarian. In special cases, this test may also be used in herds having a high probability of infection, where the CFT has been shown to be insufficient in the detection of infected animals.

3. Application – The CT must be applied only by a full-time State or Federal regulatory veterinarian who has had special training in its use. The injection site in the mid-cervical region is prepared by clipping (Number 40 blade or shaver head) a 7.8 centimeter (3 inch) square area. Adequate facilities must be available for restraint of animals being tested. Any skin abnormalities that may influence the reading of the test should be noted on the official test report.

The injection is made in the middle of the clipped area. A distinct bleb should be noted at the site when the injection is made properly (Figure 2). Marking the injection site before injecting by drawing a 12 mm (0.5 inch) circle with a magic marker may facilitate finding small responses upon reading the test.

4. Reading the CT – Palpate the injection site 72 ( $\pm$  6) hours after injection. Palpation requires that the skin be grasped so as to cause a fold over the injection site and then carefully checked for any swelling by running the thumb and forefingers of the opposite hand back and forth on either side of the fold. Any response is considered positive.
5. Recording responses – Although the size of responses is not a factor in classification, all responses to the CT should be estimated in millimeters and recorded.
6. Interpretation of the CT and classification of animals – Any detectable response will be considered evidence of *M. bovis* infection and will result in the reactor classification. There are no exceptions.
7. Retest – TB exposed animals that are negative to the CT may be retested at intervals greater than 60 days. Alternate sides of the neck are used each time. The test may not be applied to an animal within 60 days of a prior tuberculin injection. When no lesion reactors are found on two 60-day consecutive cervical tests, revert to the caudal fold test. Animals with responses to the caudal fold test may be retested with the comparative-cervical test only upon recommendation of the Regional Tuberculosis Epidemiologist and with TB staff concurrence.



Figure 2. This picture shows the proper location and injection technique for the CT in cattle and bison.

### C. CCT

1. Definition – The CCT is the intradermal injection of 0.1 ml each of biologically balanced USDA PPD Bovine and USDA PPD Avian tuberculins at premeasured sites on the neck of the bovine with observation, palpation, and postinjection measure at  $72 \pm 6$  hours.
2. Use – The CCT is used as a supplemental test on cattle and bison found suspect on the presumptive test. Its function is to clarify whether the probable cause of tuberculin sensitivity in the suspect is infection with *M. bovis*. This test is not to be used in known infected *M. bovis* herds except after an assessment concludes that the likelihood of infection remaining in such herds is small.
3. Application – This test must be conducted by a full-time State or Federal regulatory veterinarian who has been officially trained to conduct this procedure. Such veterinarians must appear on a list of those approved to conduct the CCT. The injection may be applied within 10 days, or at least 60 days, following injection of the presumptive tuberculin test in cattle and bison. Subsequent retests, if needed, will be at intervals of no less than 60 days for cattle and bison.

The upper site is about 10 centimeters (4 inches) below the crest of the neck, and the lower site is 12.5 centimeters (5 inches) below the upper site. Each site is prepared by clipping (Number 40 blade) an 8 centimeter (3 inch) square area. Clipping is necessary to identify the injection site, even on cattle and bison with very short hair.

Prior to injection, a fold of skin at the center of each clipped site is lifted as a fold and the fold measured with approved calipers. The measurement must be recorded to the nearest 0.5 millimeter in the appropriate column on VS Form 6-22C, "Tuberculin Test Record (Special)."

Balanced PPD tuberculins are intradermally injected; 0.1 ml avian in the upper site and 0.1 ml bovine in the lower site. It is important to inject the test sites in the same order on each animal to formulate a habit which will aid in reducing errors. (Remember: avium over bovine)

Use identical types of syringes and needles for each tuberculin injection. Nevertheless, make sure that the avian and bovine tuberculins never mix by using separate syringes and needles for each type of tuberculin injected.

4. Reading the test – Each injection site must be observed (in good light) and palpated  $72 (\pm 6)$  hours after injection. The point of greatest response, or the center of the clipped site in the case of no response, is lifted and measured with the same calipers used to measure the normal skin.

Both pre- and post-injection measurements must be made by the same veterinarian. In extreme circumstances, and with prior approval of the AVIC and/or State official, the test may be read by another regulatory veterinarian who is approved to conduct the test.

The pre-injection measurements, under such circumstances, may be of non-affected skin adjacent to the response. If at all possible, such tests should be canceled and repeated after 60 days.

5. Recording responses – All measurements are recorded to the nearest 0.5 mm in the specified column of the test record, VS Form 6-22C, “Tuberculin Test Record (Special).”

The pre-injection measurement of each animal is then subtracted from the post-injection measurement and the difference recorded for each test site in the specified column of VS Form 6-22C.

After subtracting the measurements, each animal tested will have a skin thickness difference value for both the avian and bovine PPD tuberculin injection sites. These two values are then represented by a single point for each animal on VS Form 6-22D, “Comparative Cervical Tuberculin Test Results” (scattergram).

6. Interpretation of the test – The completed scattergram, along with other pertinent facts known about the animal and herd, serve as a basis for interpreting the test and classifying animals tested.

Tests are interpreted by considering herd and animal history, as well as presumptive test results. The goal of interpretation is to determine the likelihood that the skin test response in any individual animal is a result of infection with *M. bovis*.

In general, each animal must be classified according to the zone into which the test results are plotted on the scattergram VS Form 6-22D: Negative for *M. bovis* (N), Suspect (S), or Reactor (R). Animals which plot on a boundary line on the VS Form 6-22D are generally classified in the more severe category of classification. Animals plotting in the suspect zone on two successive CCT shall be classified reactor.

Animals responding to the presumptive test that were found negative or suspect to the CCT shall be reclassified as reactors when *M. bovis* infection is confirmed in the herd.

An animal responding in the reactor zone, or responding twice in the suspect zone, may be classified as suspect, provided that there has been no known association of the herd with *M. bovis*. Such a reclassification is only at the discretion of the DTE or RTE. Animals classified as suspect in these situations must be moved directly to slaughter under permit. The postmortem examination shall be witnessed by a State or Federal animal health veterinarian in addition to the attending FSIS or State meat inspection veterinarian. Specimens, to include any tissue with granulomatous appearing lesions and representative head and thoracic lymph nodes, must be submitted for laboratory examination.

If such suspects fail to disclose gross evidence of bovine TB, are negative on histopathology and bacteriological cultures for *M. bovis*, and a complete epidemiologic investigation, including a herd test of all eligible animals, fails to disclose evidence of bovine TB or exposure thereto, the herd, with the concurrence of the DTE and the RTE, may be considered free of bovine TB. Any additional exceptions by the DTE and the RTE may be made only with the concurrence of the National Tuberculosis Epidemiologist.

7. Retest – An animal classified as suspect on the basis of the CCT may be retested once with the CCT no sooner than 60 days after the first CCT injection.

An animal that plots in the suspect zone on two consecutive CCT should be classified as a reactor (R), unless classified as a suspect by the DTE and handled as per #6 above.

#### D. Interferon Gamma Assay (Bovigam TM)

1. Use – The interferon gamma assay is used as a supplemental test on cattle found suspect on the presumptive test. Its function is to clarify whether the probable cause of tuberculin sensitivity in the suspect is infection with *M. bovis*.

The test may be used in parallel with the CCT or as a replacement for the CCT at the discretion of a DTE with the concurrence of the RTE. In TB affected herds that have not been depopulated, the Bovigam TM test may be used in parallel with the CFT for the identification of cattle to be removed as part of a test-and-slaughter herd plan to eliminate TB from the herd.

2. Application – As explained in VS Memorandum [552.28](#), only specifically approved individuals may conduct the interferon gamma test. At the time the CFT is read, collect blood from CFT responders via clean venipuncture in a heparinized (green-topped) tube. Only green-topped (heparin) tubes can be used as other anti-coagulants interfere with this test. One full tube per animal (6 ml) is necessary for testing. Collected blood must be kept between 45°F

and 65°F until it arrives at the lab. Use ice (gel) packs, depending on environmental conditions, to maintain temperature during storage and transport. Shipping containers should contain adequate cushioning material to prevent breakage and provide insulation of shipped samples.

3. Laboratories approved to conduct the Bovigam™ test must comply with requirements in VS Memorandum No. [552.5](#), Approval of Laboratories to Conduct Diagnostic Tests for Bovine Tuberculosis. Laboratories experienced in conducting the test, provided they have the approval of the RTE, are authorized to purchase the test kits and conduct the test with a positive sample control. The Director of the National Veterinary Services Laboratories (NVSL) will maintain a list of laboratories approved to conduct diagnostic tests for bovine TB. The list will be distributed annually to the Regional Directors, AVIC's, National Tuberculosis Surveillance Coordinator, and VS NCAHP Bovine TB Program Coordinator, and other parties upon request.
4. Interpretation of the test – RTEs and DTEs will be responsible for the interpretation and application of test results. DTEs experienced in the use and interpretation of the test, with the approval and oversight of the RTE, are authorized to use the test in field applications as indicated here.

#### VIII. SPECIAL PROCEDURES IN HIGH RISK HERDS

TB tests in high risk herds are conducted by State and Federal Regulatory Veterinarians.

- A. Testing Source Herds of Animals Found with TB at Slaughter (i.e., VS Form 6-35 investigations).
  1. After complete investigation of animal movement records and slaughter plant records, herds conclusively determined to be the source of the cattle or bison found TB positive at slaughter should be handled as follows:
    - a. Quarantine the herd immediately.
    - b. Test entire herd with the CFT; all animals in the herd over 2 months of age.
    - c. Classify animals with responses as reactors.
    - d. Slaughter reactors and submit tissues from all such animals to NVSL as described in VS Memorandum [552.7](#).
    - e. If histopathologic examination determines there is no evidence of TB, retest with the presumptive test all animals 2 years and older in the herd after 60 days. Conduct a supplemental test on all responding

animals. If presumptive tests are all negative or supplemental testing is negative, release the quarantine and retest the herd in 1 year.

- f. If histopathologic examination determines there is evidence of TB and this evidence is further supported by subsequent Polymerase Chain Reaction (PCR) and/or culture, the herd will be considered affected and will either be:
    - (1) Depopulated with approval of the herd owner, the State Veterinarian, and the Administrator of the Animal and Plant Health Inspection Service (APHIS); or
    - (2) Retested until released from quarantine as described the UM&R, Part III, Section J, point 3.
2. All other cases where a herd is the possible source of the TB positive animal.
- a. Quarantine the herd immediately.
  - b. Test all animals in the herd 2 years of age and older with the presumptive CFT.
  - c. Conduct a supplemental test on all responding animals.
  - d. If presumptive and/or supplemental test results are negative, release the quarantine.
  - e. If the presumptive and/or supplemental tests result in reactors or suspects, then slaughter all reactors or suspects and submit tissues from all such animals to NVSL as described in VS Memorandum [552.7](#).
    - (1) If histopathologic examination determines there is no evidence of TB, retest with the presumptive test all animals 2 years and older in the herd after 60 days. Conduct a supplemental test on all responding animals. If presumptive tests are all negative or supplemental testing is negative, release the quarantine.
    - (2) If histopathologic examination determines there is evidence of TB and this evidence is further supported by subsequent PCR and/or culture, the herd will be considered affected and will either be:



- (i) Depopulated with approval of the herd owner, the State Veterinarian, and the APHIS Administrator; or
  - (ii) Retested until released from quarantine as described the UM&R, Part III, Section J, point 3.
- B. Test of Herds Implicated as a Source of Infection for an Affected Herd (i.e., VS Form 6-4a investigations).
1. Test all animals in the herd that are 2 years and older with the presumptive CFT.
  2. When this herd is the source of TB-infected animals in the affected herd, or the herd is conclusively determined to be the source of infection for the affected herd, consider classifying responders as reactors on the basis of the CFT. Otherwise, conduct a supplemental test on all responding animals.
  3. If all presumptive or supplemental tests are negative, no more testing is indicated.
  4. If there are reactors or suspects proceed as described in VIII, A, 2,a & e above.
- C. Test of Exposed Animals Acquired from an Affected Herd and Herds in Contact with these Exposed Animals (i.e., VS Form 6-4b investigations).
1. If possible, the exposed animals should be purchased for diagnostic purposes without testing. Indemnity funds are usually available for this purpose and the indemnity procedures outlined in VS Memorandum [552.32](#) should be followed to facilitate such purchases. Regardless of the presence or absence of gross lesions of TB at slaughter, specimens must be submitted to NVSL according to VS Memorandum [552.7](#). Purchasing these animals for diagnostic purposes is preferred to testing because it enables a rapid and accurate diagnosis concerning exposed animals and eliminates potential spread of TB from the exposed animals to susceptible animals in the contact herd.
  2. If purchase for diagnostic purposes is not possible, then test the exposed animals with the CT. Interpret any responder to the CT as a reactor. Never use the CCT on traced exposed animals.
    - a. If the CT of the exposed animals is negative, test the balance of the animals in the herd with the presumptive CFT followed by a supplemental test on the responders. Quarantine and retest the exposed animals that remain in the herd as if they were an affected

- herd. These animals should be retested according to the schedule in the UM&R Part III, Section J, point 3 until they are released from quarantine. Although the remainder of the herd is not quarantined or considered affected, it is recommended that the balance of the herd be tested yearly during this period. The exposed animals may be slaughtered with indemnity at any time during the testing period.
- b. If there are reactors, they should be slaughtered and tissues submitted to NVSL according to VS Memorandum [552.7](#).
  - c. If there is no evidence of TB on postmortem and histopathologic examination, conduct a CFT presumptive test on the balance of the herd followed by a supplemental test on all responders to the CFT presumptive test.
  - d. If there is evidence of TB, then handle the herd as described in VIII, A, 2.
  - e. A histopathologic lesion of TB in known exposed animals with a positive PCR result is sufficient evidence to consider the entire cattle or bison herd affected.
3. When it is not possible to identify and test all of the acquired exposed animals, or when none are still present in the herd, the presumptive CFT should be conducted on all cattle and bison in the herd that are 2 years of age and older. Offspring of acquired exposed animals and younger animals that may have associated with the acquired exposed animals should be tested regardless of age. The reactor classification may be considered for animals responding to the presumptive test after herd situation is evaluated. Otherwise, conduct a supplemental test on animals responding to the presumptive test.
    - a. If the test is negative, retest with the presumptive/supplemental test combination in 1 year. It is recommended to retest animals which are 2 years and older.
    - b. If reactors or suspects are found, proceed as in VIII, A, 2, a & e.

#### D. Retest of Quarantined Herd.

Complete depopulation of the herd is the most effective method of eliminating bovine TB. However, systems of testing TB herds and removing responding animals are used when depopulation is not an option. Unfortunately, such testing fails to rid herds of *M. bovis* in a substantial number of cases. *M. bovis* appears more difficult to eliminate from larger herds than from smaller herds. When testing is used:

1. A DTE will develop a written herd plan with the owner of the affected herd to eradicate *M. bovis*. The plan will include testing schedules, sanitation practices, replacement stock purchases, calf sales or transport, safeguards against spread of *M. bovis*, culling practices, public health considerations, and other items deemed necessary by the DTE and the owner.
2. An Informal Review Committee will be formed consisting of the State Veterinarian, the AVIC in the State of the affected herd, a RTE, an epidemiologist from the National Tuberculosis Program Staff, and a representative from the appropriate livestock industry. This committee functions:
  - a. To interact and confer with the DTE on major decisions regarding the affected herd;
  - b. To review and critique the eradication plan for the affected herd in light of the scientific and political implications of its decisions;
  - c. To evaluate the progress of the eradication plan from test results and other pertinent data. The committee should concur with changing from the “Test and Removal” stage to the “Verifying TB-free” stage; and
  - d. To suggest and review proposed experimental protocols to enhance eradication of *M. bovis* from the herd.
3. Test and removal stage

Use of a supplemental test is prohibited in this stage. All responding animals are considered reactors.

The CT is the recommended test in this stage for cattle and bison. The CFT may be used in place of the CT if the State Veterinarian does not permit the use of the CT in the herd.

- a. Test ALL animals, with removal of reactors, at 60 day or greater intervals.
- b. Slaughter reactors; submit tissues from all to NVSL according to VS Memorandum [552.7](#).
- c. When no evidence of TB is found in reactors on two or more successive tests, confer with the review committee to gain its concurrence to move to the next stage, “Verifying TB-free.” Evidence of TB in these situations is defined as compatible histopathologic lesions or an *M. bovis* culture.

4. Verifying TB-free stage
  - a. Test ALL animals with the presumptive test, followed by a supplemental test on those that respond to the presumptive test per the following schedule:
  - b. Test three times without finding a reactor or suspect on supplemental tests with intervals of at least 60 days between the first and second tests and at least 180 days between the second and third tests, followed by three annual negative herd tests prior to release of the quarantine. (See Bovine TB UM&R Part III (J)(3)).
  - c. Release quarantine.
  - d. Conduct two annual herd tests following release of the quarantine.

#### IX. CONCURRENT ANIMAL INVESTIGATIONS

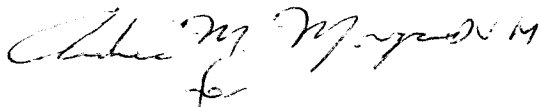
Animal species other than cattle and bison may serve as reservoirs of bovine TB. Such reservoirs may thwart schemes to eradicate *M. bovis*.

The epidemiologic investigation of an affected herd should include careful study of these species of animals. Particular attention must be paid to those species that were exposed to *M. bovis*. Appropriate actions include slaughtering, testing, and/or strict isolation of such exposed animals. The following is a brief guide for testing some of these species:

- A. Poultry – Although not susceptible to *M. bovis*, poultry infected with *M. avium* may sensitize or infect cattle or bison. (Additional information on testing poultry is found in VS Memorandum [552.16](#)).
  1. Flock should be inspected for evidence of clinical disease.
  2. Some birds should be necropsied to detect lesions of TB. The thinnest birds in the flock should be selected for this purpose.
  3. The essentials of proper management for maintenance of a TB-free flock should be discussed with the owner.
  4. Test chickens by injecting 0.05 ml PPD avian tuberculin into one wattle. Read at 48 hours by observing and palpating for any swelling. In turkeys, a wing web is the preferred site of injection.
- B. Swine – (Additional information on testing swine is found in found in VS Memorandum [552.16](#)).

1. Test swine using an intradermal injection of 0.1 ml each of PPD Bovine and/or PPD Avian in the skin on the back side of the ear at the lateral edge near the base (alternatively, the vulva of sows or anal ring of boars). Read at 48 hours by observing and palpating for any swelling.
  2. The possibility of spread of *M. bovis* between cattle, bison, and swine should always be investigated. Indemnity is allowed for depopulation of swine herds exposed to *M. bovis* infected cattle or bison (9 CFR [50](#)). Swine on affected premises depopulated for *M. bovis* should be sent to slaughter.
- C. Goats – Follow the same tuberculin testing procedures as for cattle and bison. Federal indemnity is authorized for goats on affected premises depopulated for *M. bovis* if the DTE determines their destruction will contribute to the TB eradication program. Such goats should be sent to slaughter. Owners should be advised that an accredited herd plan for dairy goats is contained in a separate VS Memorandum [552.8](#). The age for tuberculin testing goats is 6 months and over.
- D. Sheep – Follow the same tuberculin testing procedures as for cattle and bison. Federal indemnity is authorized under the conditions described above for goats. Sheep on affected premises depopulated for *M. bovis* should be sent to slaughter.
- E. Cats – The tuberculin skin test is unreliable in cats. Use postmortem or radiographic examination for diagnosis as circumstances dictate. Cats should be destroyed on premises where herds have been depopulated for *M. bovis* infection. They are susceptible and may become a source of infection to a new herd.
- F. Dogs – It is recommended that all dogs exposed to infected animals be depopulated. If depopulation is not an option, the temperature test should be conducted. The protocol for the temperature test for dogs is as follows:
1. Take the pre-injection temperature of the dog. This temperature should be in the normal range for dogs.
  2. Inject 0.75 ml PPD Bovine Tuberculin subcutaneously.
  3. Confine the dog in a comfortable place. Observe temperature of the dog at 2-hour intervals post-injection over 12 hours. A rise of 2°F is a positive response.

- G. Equidae – Horses and other equidae commonly develop non-specific responses to tuberculin. Testing is not recommended. Equidae on premises depopulated for *M. bovis* should be destroyed (if possible) or kept from direct contact with cattle or bison. Horses used to work cattle should not be housed with cattle.
- H. Camelidae (Camel, Llama, Alpaca, & Guanaco) – All species of camelidae may be tested with a tuberculin skin test in the hairless area behind the elbow, although it should be noted that the sensitivity and specificity of this test in these species is markedly lower than the skin tests for cattle and bison. Therefore, it is essential that a clinical diagnosis of TB be given some consideration even in the face of a negative tuberculin test. PPD Bovis is used at 0.1 ml dose, with observation and palpation for swelling at 48, 72, and 96 hours.
- I. Humans – Humans may be reservoirs of *M. bovis*. It is important that people who work with affected herds be evaluated by a physician for infection with *M. bovis* for their own protection and to prevent the disease from being reintroduced into the herd by these workers. Refer to a physician or public health department regarding questions about testing humans.
- J. Nonhuman Primates – There are various testing procedures for nonhuman primates. The National Institute of Health recommends intradermal testing with 0.1 ml of Old Tuberculin. Injections are made intradermally in the skin of an eyelid, the abdomen, or both. The test is read by observing and palpating for swelling at 24, 48, and 72 hours. More detailed guidelines, including a scale for interpreting the degree of the reaction are available in the “NIH Intramural Program Guidelines for the Prevention and Control of Tuberculosis in Nonhuman Primates.” A gamma interferon blood test is also available (Primagam®). This test requires 2 ml of heparinized blood which must be shipped to a laboratory equipped for conducting such tests. Turnaround time for this test is generally 2 days.



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