

Brucellosis and Bovine Tuberculosis

Program Standards

UNITED STATES DEPARTMENT OF
AGRICULTURE

Brucellosis and Bovine Tuberculosis Program Standards

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Introduction

The U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) has issued a proposed rule to consolidate the Cooperative State-Federal Brucellosis Eradication Program and the Cooperative State-Federal Tuberculosis Eradication Program into a single program.

The proposed rule, “Brucellosis and Bovine Tuberculosis; Update of General Provisions,” refers to this Program Standards document. The rule outlines performance standards for program (State and Tribal) requirements, management areas, surveillance, affected herd management and epidemiological investigations, and laboratory and diagnostic test approval, and directs persons to this document for further information regarding APHIS-approved methods to meet these standards.

Applicable provisions from the Brucellosis Eradication: Uniform Methods and Rules, effective October 1, 2003, and the Bovine Tuberculosis Eradication Uniform Methods and Rules, effective January 1, 2005, are incorporated in this guidance document. Therefore, these uniform methods and rules are obsolete.

APHIS will announce significant changes to the Program Standards document through a notice published in the *Federal Register* that provides the opportunity for public comment.

Definitions

Animal classification—program animals— brucellosis and bovine tuberculosis –

- **Negative:** Any program animal that shows no response or a negative response to an official brucellosis or tuberculosis screening test; is classified negative to an official brucellosis or tuberculosis secondary test; or is classified negative for brucellosis or tuberculosis by the epidemiologist designated by the District Director based upon history, examination of the carcass, and disease detection tests, procedures and methods as appropriate.
- **Suspect:** Any program animal that has had non-negative results to an official brucellosis or tuberculosis screening test that lead the epidemiologist designated by the District Director to determine the animal should not be classified as a reactor, but cannot be classified as free of brucellosis or bovine tuberculosis.
- **Reactor for Bovine Tuberculosis:** Any program animal that has had non-negative test results to official screening or corroboratory tests for bovine tuberculosis such that the epidemiologist designated by the District Director has determined that further action is warranted to make a final determination regarding the animal's disease status.
Reactor for Brucellosis: A program animal that had non-negative test results to official screening or corroboratory tests such that the epidemiologist designated by the District Director has determined there is a high likelihood that the animal is infected with brucellosis and a low likelihood of false positive results.

Approved diagnostic laboratory – A Federal, State, university, or National Animal Health Laboratory Network veterinary laboratory specifically recognized by the U.S. Department of Agriculture's Animal and Plant Health Inspection Service to conduct official brucellosis and bovine tuberculosis program diagnostic testing.

Bovine interferon gamma assay (BOVIGAM[®]) – An official supplemental diagnostic test for use in cattle only, that, if approved by the State animal health official and the Associate Director within the State, may be used under the direction of the epidemiologist designated by the District Director and with the concurrence of commodity health group specialists. The bovine interferon gamma assay shall only be conducted on cattle over 6 months of age on blood samples collected between 3 and 30 days after injection of bovine tuberculin for the caudal fold tuberculin test.

Calf raiser – A cattle production operation in which calves, yearlings, and other sexually immature cattle are brought together and maintained until they are of sufficient size or sexual maturity to move to their next stage of production.

Caudal fold tuberculin (CFT) test – The intradermal injection of purified protein derivative (PPD) bovine tuberculin in the tail fold of cattle or bison with reading by visual observation and palpation 72 hours (\pm 6 hours) after injection. This test must be administered only by a State, Federal or Tribal animal health official or an accredited veterinarian who is recognized as a Bovine TB Qualified Accredited Veterinarian (BTB QAV).

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Cervical tuberculin (CT) test – The intradermal injection of bovine cervical purified protein derivative (PPD) tuberculin in the cervical region of cattle or bison with reading by visual observation and palpation 72 hours (\pm 6 hours) after injection.

Comparative cervical tuberculin (CCT) test – The intradermal injection of biologically balanced bovine purified protein derivative (PPD) tuberculin and avian PPD tuberculin at separate sites in the mid-cervical area to determine the probable presence of bovine tuberculosis (*Mycobacterium bovis*) by comparing the responses to the two tuberculins at 72 hours (plus or minus 6 hours) after injection.

Epidemiologist designated by the District Director – An epidemiologist selected by the APHIS District Director, in consultation with State or Tribal animal health officials, to perform the function required of that epidemiologist.

Exposed – An animal that has had association with infected program animals, livestock, or other sources of brucellosis or bovine tuberculosis such that the epidemiologist designated by the District Director determines the animal may be infected.

Feedlot/Feedyard – A facility for assembling and feeding program animals.

Geographic separation – A minimum of 30 feet of separation, no common or shared handling facilities or equipment, no common watering or feeding equipment, and no common feed vehicles that enter the premises of herds or animals of different status. Also, if herds or animals of different status are fed by the same personnel, workers must wear different outerwear (e.g., boots and coveralls) when moving from lower status herds or animals to higher status herds or animals.

Herd test –

- For brucellosis:
 - In any area of a consistent State other than a recognized management area, testing of all sexually intact animals within a herd that are 18 months of age or older, as well as all sexually intact animals in the herd that are less than 18 months of age and were not born into the herd, except those sexually intact animals that are less than 18 months of age and originate directly from a currently accredited herd for brucellosis;
 - In any area of a provisionally consistent State other than a recognized management area, testing of all sexually intact animals within a herd that are 12 months of age or older, as well as all sexually intact animals in the herd that are less than 12 months of age and were not born into the herd, except those sexually intact animals that are less than 12 months of age and originate directly from a currently accredited herd for brucellosis.
 - In any area of an inconsistent State, or in a recognized management area for brucellosis, testing of all sexually intact animals within a herd that are 6 months of age or older, as well as all sexually intact animals in the herd that are less than 6 months of age and were not born into the herd, except those sexually intact animals that are less than 6 months of age and originate directly from a currently accredited herd for brucellosis.

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- For bovine tuberculosis:
 - In any area of a consistent State other than a recognized management area, testing of all animals within a herd that are 18 months of age or older, as well as all animals in the herd that are less than 18 months of age and were not born into the herd, except those animals that are less than 18 months of age and originate directly from a currently accredited herd for bovine tuberculosis.
 - In any area of a provisionally consistent State other than a recognized management area, testing of all animals within a herd that are 12 months of age or older, as well as all animals in the herd that are less than 12 months of age and were not born into the herd, except those animals that are less than 12 months of age and originate directly from a currently accredited herd for bovine tuberculosis.
 - In any area of an inconsistent State and in a recognized management area for bovine tuberculosis, testing of all animals within a herd that are 6 months of age or older, as well as all animals in the herd that are less than 6 months of age and were not born into the herd, except those animals that are less than 6 months of age and originate directly from a currently accredited herd for bovine tuberculosis.

High risk program animals – Disease-exposed program animals residing in a disease-affected herd that have a negative result to a screening test, or any program animals residing in a quarantine feedlot or quarantine pen. Steers and spayed heifers residing in a brucellosis-affected herd are not considered high-risk cattle. High-risk cattle may only be fed in quarantine feedlots or pens.

IDEXX *M. bovis* Antibody (Ab) test – A test used to detect the presence of serum antibodies to *M. bovis* in blood collected from cattle 3 months of age and older in TB affected herds. Blood must be collected by a State, Tribal, or Federal veterinarian or technician employed by State, Tribal, or Federal government and approved by such government to collect blood for the IDEXX *M. bovis* Ab test when directly supervised by State, Tribal, or Federal animal health veterinarians. Blood for the IDEXX *M. bovis* Ab test must be collected between 7 and 75 days after CFT injection.

Management area -- A clearly delineated geographical area in which a State or Tribe has detected brucellosis or bovine tuberculosis, has determined that there is a risk of transmission of brucellosis or bovine tuberculosis to program animals, and has taken or proposes to take measures to control the spread of the brucellosis or bovine tuberculosis within and from the area and/or to eradicate the disease within the area.

Official brucellosis vaccinate-- An animal that has been vaccinated with an official brucellosis vaccine, tattooed in a manner that specifies that animal's brucellosis vaccination status, and officially identified in accordance with the Program Standards document (see [Official brucellosis vaccination program](#)), or in another manner approved by the Administrator.

Official Brucella vaccine-- A vaccine for brucellosis that has been approved by the Administrator and produced under license of the United States Department of Agriculture.

Official test – Any test that is approved by the Administrator for determining the presence or absence of brucellosis or bovine tuberculosis in program animals and that is conducted and

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reported by an official tester. If an official test is applied to a program animal, it must be identified by means of an official eartag.

- Brucellosis: For cattle and bison, the official brucellosis tests include the brucellosis ring test, the rapid automated presumptive test, the buffered acidified plate antigen test, the card test, the fluorescent polarization assay, the heat inactivation ring test, the complement fixation test, and the rivanol test. For captive cervids, the official brucellosis tests are the buffered acidified plate antigen (BAPA) and fluorescent polarization assay (FPA). See [Diagnostic tests and interpretation for bovine brucellosis](#).
- Bovine tuberculosis: For cattle and bison, the official tuberculosis tests include the caudal fold tuberculin test (CFT), the comparative cervical tuberculin test (CCT), and the cervical tuberculin test (CT). For cattle only the bovine interferon gamma assay and the IDEXX *M. bovis* Ab serological test. For captive cervids, official tuberculosis tests include the single cervical tuberculin test (SCT), the CCT, and, for elk, red deer, white-tailed deer, fallow deer, and reindeer, the DPP[®] tests. See [Official tests for bovine tuberculosis](#).

Official tester - Any person associated with the conducting and reporting of official tests within an official testing laboratory, or any person authorized by the Administrator to conduct and report official diagnostic tests outside of the laboratory environment.

Official testing laboratory – A laboratory approved by the Administrator in accordance with 9 CFR, part 76, to conduct official tests.

Parallel testing – Two or more diagnostic test procedures applied to an animal or herd with the results being interpreted simultaneously. If one or more of the test procedures is nonnegative, the animal or herd cannot be classified as negative. This type of testing is usually performed to increase sensitivity or disease detection ability.

Postmortem examination – A gross necropsy evaluation after death of an animal, submission of tissue samples for histopathological examination, bacterial identification, and/or culture. Reports of gross necropsy findings, histopathological examinations, bacterial identification, and/or culture must be included in the herd files associated with the investigation.

Bovine tuberculosis (BTB) qualified accredited veterinarian (BTB QAV) – An accredited veterinarian in Category II who has been granted a program certification by the Administrator pursuant to 9 CFR 161.5 based on completion of an APHIS-approved orientation or training program.

Quarantine – An order under State, Tribal, or Federal authority to restrict movement of animals into or from a premises or geographical area until the disease status of the animals is determined or after a diagnosis of disease has been made by regulatory officials. A quarantine usually restricts all animals to the farm and prevents additions while disease status is being determined or after a diagnosis of disease has been made except in some instances when animals are allowed to move directly to slaughter.

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Quarantine pen – An area within a feedlot/feedyard approved by the Associate Director for assembling and feeding high risk program animals without risk of spread of brucellosis or bovine tuberculosis to other susceptible animals at the facility. Approval will be granted only after a State, Tribal, or APHIS representative inspects the area and determines that all high risk program animals are secure and geographically separated from contact with all other program animals, that there are facilities for identifying high risk program animals, and that there is no likelihood of brucellosis or bovine tuberculosis being transmitted from the area. All program animals leaving quarantine pens must only move directly to slaughter or another approved quarantine feedlot/feedyard or approved quarantine pen in accordance with established procedures for handling quarantined livestock. See [Quarantine feedlot/feedyard](#).

Quarantine feedlot/feedyard – A facility approved by the Associate Director for feeding high risk program animals without risk of spread of brucellosis or bovine tuberculosis to other susceptible animals at the facility. Approval will be granted only after a State, Tribal, or APHIS representative inspects the confined area and determines that all program animals are secure and geographically separated from contact with all other program animals, that there are facilities for identifying program animals, and that there is no likelihood of brucellosis or bovine tuberculosis being transmitted from the confined area. All program animals leaving quarantine feedlots/feedyards must only move directly to slaughter or another approved quarantine feedlot/feedyard or approved quarantine pen in accordance with established procedures for handling quarantined livestock. See [Quarantine feedlot/feedyard](#).

Regulatory veterinarian– A veterinarian employed by a State, Tribal, or Federal animal health agency.

Screening test – An official test that typically has high sensitivity and is frequently used to screen large numbers of samples at a lower cost. Samples positive on a screening test may be retested using a secondary test. Animals testing negative on screening tests are considered negative.

Secondary (corroboratory) test – An official test that typically has a high specificity that is used to verify the test status of the animal or herd that has had a non-negative screening test result. Results from secondary tests are used to classify animals as negative, suspect, or reactor.

Serial testing – Two or more diagnostic test procedures applied to an animal or herd with the results being interpreted sequentially. All test results in a series must be non-negative for the animal to be classified as positive. This type of testing is usually performed in order to increase specificity or to confirm disease.

Single cervical tuberculin (SCT) test – The intradermal injection of bovine purified protein derivative (PPD) tuberculin in the mid-cervical region in a cervid with reading by visual observation and palpation 72 hours (\pm 6 hours) following injection. This test must be administered only by a State, Federal or Tribal animal health official or an accredited veterinarian who is recognized as a Bovine TB Qualified Accredited Veterinarian (BTB QAV). Captive cervids will not be subjected to SCT retest at intervals of less than 90 days.

Source herd – The herd in which an animal resided prior to the herd in which it currently resides.

Supplemental tests – A test that has been developed to aid designated brucellosis epidemiologists in better interpreting the results of official tests, allowing trained and qualified epidemiologists a degree of variance from the diagnostic criteria included in this document. Supplemental tests are not official tests. Supplemental tests include, but are not limited to, molecular characterization techniques (Whole Genome Sequencing, DNA microsatellite testing, etc.). Only the epidemiologist designated by the District Director, in consultation with commodity health group specialists, may use and interpret results of supplemental tests.

Tests –

- **Screening test:** The screening test for bovine tuberculosis in cattle and bison is the caudal fold tuberculin test. The screening test for bovine tuberculosis in captive cervids is the single cervical tuberculin test or, for elk, red deer, white-tailed deer, fallow deer, or reindeer, the DPP[®] test. The screening tests for brucellosis include the rapid automated presumptive (RAP) test, the buffered acidified plate antigen (BAPA) test, the card test, the fluorescent polarization assay (FPA), the brucellosis ring test (BRT) and the heat inactivated ring test (HIRT).
- **Secondary (corroboratory) test:** The secondary test for bovine tuberculosis in cattle or bison is the comparative cervical tuberculin test or, for cattle only, the gamma interferon. The secondary test for bovine tuberculosis in captive cervids is the comparative tuberculin test, or, for elk, red deer, white-tailed deer, fallow deer, or reindeer, the DPP[®] test. The secondary tests for brucellosis include the fluorescent polarization assay (FPA), the complement fixation (CF) test, and the rivanol test.
- **Additional tests or variations to the test requirements must be determined by the epidemiologist designated by the District Director in conjunction with commodity health group specialists and approved by the Administrator.**

Acronyms

APHIS – Animal and Plant Health Inspection Service

ARS – Agricultural Research Service

AD – Associate Director

BAPA – Buffered acidified plate antigen

BTB – Bovine tuberculosis

BRT – Brucellosis Ring Test

BTB QAV – Bovine tuberculosis qualified accredited veterinarian

CCT – Comparative cervical tuberculin test

CDC – Centers for Disease Control and Prevention

CEAH – Centers for Epidemiology and Animal Health

CHG-Commodity Health Group

CLIA – Clinical Laboratory Improvement Amendments

CT – Cervical tuberculin test

CVB – Center for Veterinary Biologics

CFR – *Code of Federal Regulations*

CFT – Caudal fold tuberculin test

FFPE – Formalin fixed paraffin embedded

FPA – Fluorescence polarization assay

FPT – First point testing

FSIS – Food Safety and Inspection Service

IATA – International Air Transport Association

ICVI – Interstate certificate of veterinary inspection

IES – Investigative and Enforcement Services

MOU – Memorandum of understanding

NCAHP – National Center for Animal Health Programs

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NGL – No Gross Lesions

NIH – National Institutes of Health

NSU – National Surveillance Unit

NVSL – National Veterinary Services Laboratories

OD – Optical density

PCR – Polymerase chain reaction

PPD – Purified protein derivative

RAP – Rapid automated presumptive

SAHO – State animal health official

SCT – Single cervical tuberculin test

SNP – Single nucleotide polymorphism

SPT – Standard plate test

STT – Standard tube test

TB – Tuberculosis

USAHA – United States Animal Health Association

VNTR – Variable number tandem repeat

VS – Veterinary Services

WGS – Whole Genome Sequencing

WS – Wildlife Services

Element 1: Program Requirements

Regulatory authority: 9 CFR 76.2, animal health plan requirements; 9 CFR 76.2(g), compliance reviews; 9 CFR 76.3, State or Tribal classifications; and 9 CFR 76.4, reporting requirements.

Related VS policy: VS Memorandum 515.1, Guidelines for Veterinary Services Program and Station Reviews, (3/23/09)

Background

The [Animal health plan](#) is used as a key determinant when Animal and Plant Health Inspection Service (APHIS) classifies States and Tribes with a status for the brucellosis and bovine tuberculosis programs. These plans:

- Describe a State's or Tribe's brucellosis and bovine tuberculosis program activities.
- Document a State's or Tribe's compliance with the Federal regulations, policies, and performance standards for the brucellosis and bovine tuberculosis programs.

We will make approved animal health plans publicly available in conjunction with published notices in the *Federal Register*. We may also post these plans on our Web site.

[State and Tribal classifications](#) are a foundational component of the brucellosis and bovine tuberculosis programs. They serve two primary purposes:

- Provides a structure that ensures consistency in program implementation and compliance across States and Tribes.
- Provides animal health information and documents disease status (i.e., risk) for both domestic and international trading partners.
- APHIS will classify States and Tribes using a three-tiered system. We will designate a separate status for bovine tuberculosis and brucellosis as follows:
 - Consistent.
 - Provisionally consistent.
 - Inconsistent.
- APHIS will consider two factors when classifying States and Tribes:
 - Implementing an approved animal health plan (See [Animal health plan](#)).
 - Reporting to document continual adherence to the animal health plan and describe occurrences of disease (See [Reporting](#)).

APHIS may conduct [Program compliance](#) reviews to evaluate a State's or Tribe's compliance with its animal health plan. We may also conduct reviews when considering re-designating a State's or Tribe's brucellosis or bovine tuberculosis program status or evaluating a State's or Tribe's request for establishing, changing, or terminating a recognized management area. APHIS will evaluate State and Tribe activities to ensure that they are fully

implementing and complying with their plans as well as with Federal regulations, policies, and performance standards for brucellosis and bovine tuberculosis.

1.1 Animal health plan

1.1.1 Submission requirements and evaluation criteria

The animal health plan must contain all of the categories of information described in 9 CFR 76.2 for the disease of interest. An outline of submission requirements for each category is provided below.

- A template for developing the animal health plan is available (see
- [Test interpretation and animal classification](#);
- ANY increase in caudal fold thickness at the site of injection -- either observed or palpated -- is considered a positive test result (i.e., a response).
 - Classify the animal with a positive test result (i.e., a response) as a suspect, unless the reactor classification is indicated.
 - Epidemiologists designated by the District Director and CHG specialists may classify a CFT-responding animal as a reactor when that classification is indicated.
 - For example, the reactor classification may be indicated when an animal or the herd in which it resides may have been or was exposed to a bovine tuberculosis-affected herd or an animal infected with *M. bovis*.
- Specific instructions for reporting:
 - **The official tester must IMMEDIATELY (same working day) report ALL positive test results (i.e., responses) to the State or Tribal Veterinarian or the AD of the State or Tribe where the animal is located.**
 - Complete and submit the VS Form 6-22, “Tuberculosis Test Record”.
 - For the CFT test results:
 - Record a plus (+) sign in the first column under “Results” labeled “Size” for any increase in caudal fold thickness or other inflammatory response at the site of the injection.
 - For the second column under “Results” labeled “NRS”, mark with an N (negative) when no response was detected or an S (suspect) when a (+) in the size column indicates that response did occur.
- Retesting an animal
 - Animals that are suspects on the CFT must be retested with a secondary test to determine their final classification.
 - The CFT may not be applied to bovines within 60 days of any prior tuberculin injections.

8.4.3.2 Single cervical tuberculin (SCT) test

- Test description:

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- The SCT test is an official test for bovine tuberculosis in cervids.
- The SCT test is based on observation of a delayed-type hypersensitivity (Type IV) immune response. Animals infected with or previously exposed to mycobacterial species exhibit an inflammatory response at the site of an intradermal injection of PPD tuberculin.
- Appropriate use of the test:
 - The SCT is used as a screening test for bovine tuberculosis infection in cervids.
- Official testers:
 - State, Federal, or BTB QAVs for bovine tuberculosis may apply the SCT test.
 - BTB QAVs may NOT apply the SCT test in TB affected captive cervid herds or captive cervid herds under epidemiological investigations. Only State, Tribal, or Federal regulatory veterinarians may perform the SCT test in these herds.
- Sample type:
 - Intradermal skin test
- Performing the test:
 - Properly restrain the animal. Proper restraint of cervids is necessary to apply the SCT and to ensure the safety of the official tester(s).
 - The injection site for the SCT test is in the mid-cervical region.
 - Prepare the injection site by clipping a 6 centimeter (2 ½ inch) square area.
 - Note any abnormalities found near the injection site on the official test record so that such abnormalities will not be mistaken for tuberculin responses during test observation.
 - Mark the injection site before injecting by drawing a 12 mm (0.5 inch) circle with a permanent marker. This may enable the tester to find small responses when reading the test.
 - Inject 0.1 ml PPD Bovine Tuberculin intradermally in the middle of the clipped area.
 - Observe and palpate the injection site 72 (± 6) hours after injection.
 - Properly restrain the animal.
 - The veterinarian who makes the tuberculin injection must be the one who “reads” (determines) the results of the test.
 - Grasp the skin so as to cause a fold of skin at the injection site. Palpate the injection site by running the thumb and forefinger of the opposite hand back and forth along the fold. Palpation which is limited to running the fingertips over the skin surface is not acceptable. Test interpretation and animal classification:

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- ANY increase in skin thickness at the site of injection – either observed or palpated – is considered a positive test result (i.e., a response).
- Classify the animal with a positive test result (i.e., a response) as a suspect, unless the reactor classification is indicated.
 - Epidemiologists designated by the District Director or CHG specialists may classify a SCT responding animal as a reactor when that classification is indicated.
 - For example, the reactor classification may be indicated when an animal or the herd in which it resides may have been or was exposed to a bovine tuberculosis-affected herd or an animal infected with *M. bovis*.
- Specific instructions for reporting:
 - **The official tester must IMMEDIATELY (same working day) report ALL positive test results (i.e., responses) to the State/Tribal Veterinarian or the AD of the State or Tribe where the animal is located.**
 - Complete and submit the VS Form 6-22, “Tuberculosis Test Record”.
 - For the SCT test results:
 - Record the estimated increase in skin thickness (in millimeters) in the first column under “Results” labeled “Size” for any increase in caudal fold thickness or other inflammatory response at the site of the injection.
 - For the second column under “Results” labeled “NRS”, mark with an “N” (negative) when no response was detected or an “S” (suspect) when a the size column indicates that response did occur.
- Retesting an animal:
 - Animals that are suspects on the SCT must be retested with a secondary test to determine their final classification.
 - The SCT may not be applied to a captive cervid within 90 days of any prior tuberculin injections.
 - Use alternating sides of the neck for subsequent tests.

8.4.3.3 Cervical tuberculin (CT) test

- Test description:
 - The cervical tuberculin (CT) test is an official test for bovine tuberculosis in cattle and bison.
 - The CT test is based on observation of a delayed-type hypersensitivity (Type IV) immune response. Animals infected with or previously exposed to mycobacterial species exhibit an inflammatory response at the site of an intradermal injection of PPD tuberculin.
- Appropriate use of the test:

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- The CT is a screening test that is limited to use only in cattle and bison known to have been exposed to *M. bovis*.
- The CT is the only test approved for use on exposed animals acquired from a bovine tuberculosis-affected herd (VS Form 6-4b investigations).
- The test may also be used for testing bovine tuberculosis-affected herds managed under a test-and-remove protocol.
- The CHG specialist may recommend the use of the CT in specific animals or herds under investigation.

- Official testers:
 - Only approved State, Tribal, or Federal veterinarians may apply the CT test.

- Sample type:
 - Intradermal skin test

- Performing the test:
 - Properly restrain the animal.
 - The injection site for the CT test is in the mid-cervical region.
 - Prepare the injection site by clipping a 7.8 centimeter (3 inch) square area.
 - Note any abnormalities found near the injection site on the official test record so that such abnormalities will not be mistaken for tuberculin responses during test observation.
 - Mark the injection site before injecting by drawing a 12 mm (0.5 inch) circle with a permanent marker. This may enable the tester to find small responses when reading the test.
 - Inject 0.1 ml of bovine cervical test PPD tuberculin (PPD Bovine, Cervical, 2mg/ml) intradermally in the middle of the clipped area.
 - Observe and palpate the injection site 72 (\pm 6) hours after injection.
 - Properly restrain the animal.
 - The veterinarian who makes the tuberculin injection must be the one who “reads” (determines) the results of the test.
 - Grasp the skin so as to cause a fold of skin at the injection site. Palpate the injection site by running the thumb and forefinger of the opposite hand back and forth along the fold. Palpation which is limited to running the fingertips over the skin surface is not acceptable.

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Figure 5 Proper location and injection technique for the CT in cattle and bison



- Test interpretation and animal classification:
 - ANY detectable change, palpable or visual, at the site of injection -- either observed or palpated -- is considered a positive test result (i.e., a response).
 - Animals with a positive test result (i.e., a response) **MUST** be submitted for post-mortem evaluation.
 - Recall that this test uses a more concentrated dosage of tuberculin to increase the sensitivity of the test.
 - All responders are taken to necropsy or slaughter for further evaluation.
 - The animal may be classified as either a suspect or a reactor. Consult with the epidemiologist designated by the District Director or the CHG specialist to determine which classification is indicated.

- Specific instructions for reporting:

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- **The official tester must IMMEDIATELY (same working day) report ALL positive test results (i.e., responses) to the State/Tribal Veterinarian or the AD of the State or Tribe where the animal is located.**
- Complete and submit the VS Form 6-22, “Tuberculosis Test Record”.
- For the CT test results:
 - Although the size of responses is not a factor in classification, record the estimated increase in skin thickness (in millimeters) in the first column under “Results” labeled “Size” for any increase in caudal fold thickness or other inflammatory response at the site of the injection.
 - For the second column under “Results” labeled “NRS,” mark with an “N” (negative) when no response was detected or an “R” (suspect) when a the size column indicates that a response did occur.
- Retesting an animal
 - Testing interval \geq 60 days.
 - The CT may not be applied to an animal within 60 days of any prior tuberculin injection.
 - Use alternating sides of the neck for subsequent tests.
 - Revert to using the CFT test after two consecutive CT tests fail to reveal lesioned reactors.

8.4.3.4 IDEXX *M. bovis* Ab Test

- Test description:
 - The IDEXX *M. bovis* Ab Test is an official test for bovine tuberculosis in TB affected cattle herds during the test and removal phase of a herd management plan only.
 - The IDEXX *M. bovis* Ab Test is a test for serum antibodies to *M. bovis*.
- Appropriate use of the test:
 - The IDEXX *M. bovis* Ab Test may be used during the removal phase of test-and-remove management plans in TB-affected cattle herds. Other uses will be considered on a case-by-case basis.
 - The IDEXX *M. bovis* Ab Test is approved for cattle 3 months of age and older.
 - Use of the IDEXX *M. bovis* Ab Test is at the discretion of the epidemiologist designated by the District Director or Area Epidemiology Officer (AEO) with approval required by the commodity health group specialist.
- Official testers:
 - State, Tribal, or Federal Regulatory veterinarians will collect blood samples for the IDEXX *M. bovis* Ab Test.
 - Technicians employed by a State, Tribal, or Federal government and approved by such government may collect blood for the IDEXX *M. bovis* Ab Test when directly supervised by State, Tribal, or Federal animal health veterinarians.
- Sample type:

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- Serum
 - Blood samples for IDEXX *M. bovis* Ab Testing must be collected no sooner than 7 days after the CFT is injected and no more than 75 days after the CFT is injected. To ensure rapid identification and removal of potentially infected animals, VS recommends collecting blood samples between 7 and 14 days after the CFT is injected.
- Attach a copy of the completed VS Form VS 6-22 (Tuberculosis Test Record).
- Test interpretation and animal classification:
 - The epidemiologist designated by the District Director will classify the animals interpreting the CFT and IDEXX *M. bovis* Ab Tests in parallel.
 - Animals negative on the CFT and IDEXX *M. bovis* Ab Test, SIP ratio < 0.3, should be classified as negative.
 - Animals nonnegative on the CFT or the IDEXX *M. bovis* Ab Test, S/P ratio \geq 0.3, must be examined postmortem for evidence of TB.

8.4.4 Secondary tests for bovine tuberculosis

8.4.4.1 Comparative cervical tuberculin (CCT) test

- Test description:
 - The comparative cervical tuberculin (CCT) test is an official test for bovine tuberculosis in cattle, bison, and cervids.
 - The CCT test is based on observation of a delayed-type hypersensitivity (Type IV) immune response. It distinguishes between cell-mediated immune responses to *M. avium* from those to *M. bovis* by comparing the animal's responses to intradermal injections of both types of PPD tuberculin.
- Appropriate use of the test:
 - The CCT is used as a secondary test for bovine tuberculosis infection in cattle, bison, and captive cervids classified as a suspect based on a positive test result (i.e. response) to a tuberculin skin test during screening.
 - This test is not to be used during the removal stage of testing in known infected *M. bovis* herds. Exceptions must be approved by the epidemiologist designated by the District Director in conjunction with the CHG specialist.
- Official testers:
 - Only approved State, Tribal, or Federal veterinarians may apply the CCT test.
- Sample type:
 - Intradermal skin test
- Performing the test:
 - Properly restrain the animal.

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- The injection sites for the CT test are in the mid-cervical region.
 - Prepare two injection sites. 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.
 - Clip an 8 centimeter (3 inch) square area at each site.
 - Prior to injection, lift a fold of skin at the center of each clipped site and measure the fold to the nearest 0.5 millimeters with approved calipers.
 - Record the measurements on VS Form 6-22C, “Tuberculin Test Record (Special)”, as described below.
- Inject the balanced PPD tuberculins intradermally as follows:
 - 0.1 ml PPD avian in the upper site
 - 0.1 ml PPD bovine in the lower site.
 - Inject the test sites in the same order on each animal to reduce interpretation errors. (Remember: avium over bovine)
 - Use separate syringes and needles for each type of tuberculin to ensure that the avian and bovine tuberculins never mix.
 - Use identical types of syringes and needles for each tuberculin injection.
- Palpate the injection site and measure skin thickness 72 (\pm 6) hours after injection.
 - The veterinarian who makes the tuberculin injection must be the one who performs the pre- and post-injection measurements and “reads” (determines) the results of the test.

NOTE: If a deviation from this policy was approved in advance and documented in writing by the AD and State Veterinarian, the test may be read by another regulatory veterinarian who is approved to conduct the CCT. In this situation, the pre-injection measurements may be of non-affected skin adjacent to the response.

- 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 approved calipers used to measure the normal skin.
- Test interpretation and animal classification:
 - The goal of interpretation at this stage of testing is to determine the likelihood that a positive result on a skin test (i.e., a response) in an individual animal is the result of infection with *M. bovis*. The interpretations of the results of the CCT test should consider pertinent herd and animal history, as well as presumptive test results for the animal.

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- Complete and submit the VS Form 6-22C, “Tuberculin Test Record (Special).” For both the avium and bovine PPD injection sites:
 - Record pre- and post-injection measurements to the nearest 0.5 mm in columns labeled “Normal” and “72 Hours.”
 - Record the difference after subtracting the pre-injection measurement from the post-injection measurement in the column labeled “increase.”
 - Complete and submit the VS Form 6-22D, “Comparative Cervical Tuberculin Test Results.”
 - Plot a single point representing the skin thickness difference value for both the avian (y-axis) and bovine (x-axis) PPD tuberculin injection sites for a single animal.
 - Use completed form VS Form 6-22D, Comparative Cervical Tuberculin Test Results (i.e., the scattergram) as a basis to classify the animal.
 - In general, classify each animal according to the zone into which its test results are plotted on the scattergram: negative for *M. bovis* (N), suspect (S), or reactor (R).
 - Use the bovine boundary lines to classify reindeer tested with the CCT.
 - Use the more severe category of classification for animals with a test result that is plotted on a boundary line on the scattergram.
 - Record the classification of the animal on the VS Form 6-22C as:
 - “N” for negative *M. bovis*
 - “S” for suspect
 - “R” for reactor
 - The following information about the herd or animal bovine tuberculosis testing history will influence the interpretations of the results of the CCT test and the classification of the animal.
 - Classify the following animals as reactors:
 - Animals with test results that plotted in the reactor zone on any CCT tests.
 - Animals with test results that plotted in the suspect zone on two successive CCT tests.
 - Animals that responded to the screening test and were subsequently found to be negative or suspect to the CCT test if *M. bovis* infection is confirmed in the herd.
 - The epidemiologist designated by the District Director or CHG specialist may reclassify an animal responding in the reactor zone, or responding twice in the suspect zone, as a suspect.
 - Animals classified as a suspect MAY be submitted for post mortem evaluation.
 - Animals reclassified as a suspect by the epidemiologist designated by the District Director or the CHG specialist MUST be submitted for post mortem evaluation.
 - Animals classified as a reactor MUST be submitted for post mortem evaluation.
- Specific instructions for reporting
 - **The official tester must IMMEDIATELY (same business day) report ALL positive test results (i.e., responses) to the State/Tribal Veterinarian or the AD of the State or Tribe where the animal is located.**

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- Retesting an animal
 - If used in cattle or bison, the CCT test must be applied within 10 days of the injection date of a CFT test. Otherwise, it must be administered more than 60 days after the date of injection of a CFT test with a positive test result (i.e., a response).
 - If used in cervids, the CCT test must be applied within 10 days of the injection date of a SCT test. Otherwise, it must be administered more than 90 days after the date of injection of a SCT test with a positive test result (i.e., a response).
 - Use the opposite side of the neck from SCT test injection, especially if the two tests are applied within 10 days of each other.
 - Testing interval ≥ 60 days in cattle and bison; ≥ 90 days in cervids.
 - The CCT test may not be applied to cattle or bison within 60 days of any prior tuberculin injection in cattle or bison or within 90 days for cervids.
 - An animal classified as suspect on the basis of the CCT test may be retested once with the CCT. The second CCT test must occur no sooner than 60 days after the first CCT test injection in cattle and bison and 90 days after the first CCT test injection in cervids.
 - An animal that plots in the suspect zone on two consecutive CCT tests should be classified as a reactor (R), unless classified as a suspect by the epidemiologist designated by the District Director or the CHG specialist, provided that there has been no known association of the herd with *M. bovis*.

8.4.4.2 Bovine gamma interferon test

- Test description:
 - The bovine gamma interferon test (BOVIGAM[®]) is an official test for bovine tuberculosis in cattle greater than 6 months of age.
 - The bovine gamma interferon test (BOVIGAM[®]) distinguishes between cell-mediated immune responses to *M. avium* from those to *M. bovis* by comparing the relative amount of interferon gamma that an animal's macrophages produce in response to both types of PPD tuberculin in an ELISA format.
 - Cattle 6 months of age and younger should not be tested using the gamma interferon test because non-specific reactivity to bovine tuberculin occurs, resulting in false positive responses.
- Appropriate use of the test:
 - The bovine gamma interferon test (BOVIGAM[®]) is used as a secondary test for bovine tuberculosis infection in cattle classified as a suspect based on a positive test result (i.e. response) to a tuberculin skin test during screening.
 - The State or Tribal Veterinarian and the AD must approve the use of the bovine gamma interferon test (BOVIGAM[®]) as a replacement for the CCT.
 - The bovine gamma interferon test (BOVIGAM[®]) is NOT approved for official use in other species.
 - The bovine gamma interferon test (BOVIGAM[®]) is NOT approved for use as a screening test.
 - The bovine gamma interferon test (BOVIGAM[®]) may also be used in parallel with the CFT test in bovine tuberculosis-affected herds that are managed under a test-and-

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removal herd plan at the discretion of the epidemiologist designated by the District Director with the concurrence of the CHG specialist.

- Official testers:
 - Approved State/Tribal or Federal veterinarians may collect blood from cattle.
 - Approved animal health technicians employed by a State, Tribal, or Federal government may collect blood from cattle when approved to do so by such governments and directly supervised by State, Tribal, or Federal veterinarians that are on-site at the time of testing.
 - BTB QAVs for bovine tuberculosis may collect blood from cattle in States or Tribes, when approved by the State or Tribal Veterinarian and the AD.
 - Only approved laboratories that utilize standard operating procedures approved by NVSL may perform the bovine gamma interferon test (BOVIGAM[®]) as an official test for bovine tuberculosis in cattle.

- Sample type and collection:
 - Collect 6 ml or more of whole blood in a heparinized (i.e., green-topped) blood tube. Other anti-coagulants interfere with this test.
 - Collect blood 3 to 30 days after CFT injection from CFT test responders (i.e. animals classified as suspects) at the time the CFT test is read. It is critical to maintain blood at at $22^{\circ}\pm 5^{\circ}$ C ($71.6^{\circ}\pm 9^{\circ}$ F) during handing and shipping. Contact the approved laboratory or NVSL for specific shipping instructions.

- Test interpretation:
 - **Negative:** Samples with a negative result are reported as being negative for *Mycobacterium bovis*. A sample is considered negative if the OD value of either the nil antigen or the avian antigen subtracted from the OD value of the bovine antigen gives a value less than 0.1. Negative = bovine antigen OD - nil antigen OD <0.1 or bovine antigen OD – avian antigen OD <0.1
 - **Positive:** Samples with a positive result are reported as being positive for *Mycobacterium bovis*. A sample is considered positive if the OD value of the nil antigen subtracted from the OD value of the bovine antigen gives a value greater than or equal to 0.1, and the OD value of the avian antigen subtracted from the OD value of the bovine antigen gives a value greater than or equal to 0.1. Positive = bovine antigen OD – nil antigen OD \geq 0.1 and bovine antigen OD – avian antigen OD \geq 0.1
 - **Nonviable:** Nonviable samples are reported as being nonviable. For a sample to be considered viable, the OD value of the pokeweed mitogen well for the sample must be at least 0.1 greater than the OD value of the nil antigen well for that sample. Valid sample = pokeweed OD \geq nil antigen OD + 0.1
 - **Not Tested:** Sample did not meet previously described criteria for testing.

- Animal classification:
 - The epidemiologist designated by the District Director, in consultation with the CHG specialist, interprets the test results described to classify the animal.

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- Generally, an animal with a positive result on a routine test for sale, show, movement, or milk ordinance in AF zones, the bovine gamma interferon test (BOVIGAM®) (where positive and negative controls meet requirements) are classified as:
 - An animal with a positive result where bovine antigen OD – avian antigen OD is between 0.1 and 0.4999 should be classified as a suspect.
 - An animal with a positive result where bovine antigen OD – avian antigen OD is ≥ 0.5000 should be classified as a reactor. The epidemiologist designated by the District Director or the CHG specialist may reclassify the animal as suspect but the animal must be evaluated for tuberculosis postmortem.
 - If the initial gamma interferon test is not tested or nonviable:
 - Test with the CCT, if still prior to the 10 day window from the CFT, and use the CCT results as the official results, or:
 - Retest with the gamma interferon if past the 10 day window and before 30 days from the CFT.
 - If the gamma interferon test is not tested or nonviable twice, the animal remains a suspect and may be retested with CCT after waiting 60 days from the CFT.
 - Information about the herd's or animal's bovine tuberculosis testing history and other risk factors may influence the interpretation of the test results and the classification of the animal.
 - Animals with positive test results on two successive gamma interferon tests should be classified as a reactor. However, the epidemiologist designated by the District Director or the CHG specialist may reclassify this animal as a suspect, if indicated, but it must be evaluated postmortem.
 - The epidemiologist designated by the District Director or the CHG specialist may classify an animal as a suspect based on a different cut-off value for a positive result, when deemed appropriate.
 - The epidemiologist designated by the District Director or the CHG specialist may classify an animal as a reactor based on a relatively high OD value or based on a positive test result in light of herd or animal test history.
 - Specific instructions for reporting:
 - **The official tester must IMMEDIATELY (same working day) report ALL positive test results to the State/Tribal Veterinarian or the AD of the State or Tribe where the animal is located.**
 - Retesting an animal:
 - The bovine gamma interferon test (BOVIGAM®) must only be used for blood samples collected between 3 and 30 days after the injection date of a CFT test.
 - The epidemiologist designated by the District Director or the CHG specialist may approve a retest for animals with a positive result on one bovine gamma interferon test (BOVIGAM®) so long as the blood sample for the second test is collected within 30 days of the injection date of a CFT test.
- 8.4.4.3 *Reclassification of CCT and Gamma interferon suspects as negative*
- CCT test suspects must be retested negative by the CCT test prior to reclassifying such suspects as negative.

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- Gamma interferon suspects must be retested negative by the gamma interferon test prior to reclassifying such suspects as negative.
- Gamma interferon suspects may not be retested with the CCT.
- CCT suspects may not be retested with the gamma interferon.
- When both CCT and Gamma interferon are used on the same CFT suspect animal, they must be interpreted in parallel.

8.4.5 CervidTB DPP test

- Test description:
 - The CervidTB DPP is an official test for bovine tuberculosis in elk, red deer, white-tailed deer, fallow deer, and reindeer.
 - The CervidTB DPP is a serological test that tests test for antibodies to TB in the serum
- Appropriate use of the test:
 - The DPP test is used both as a screening and a secondary test for bovine tuberculosis infection in the approved cervid species.
 - The CervidTB DPP may be used in parallel with the SCT and CCT tests but a positive DPP or CCT test will classify the animal as a suspect.
- Official testers:
 - Approved State/Tribal or Federal veterinarians may collect blood from cervids.
 - Approved animal health technicians employed by a State, Tribal, or Federal government may collect blood from cervids when approved to do so by such governments and directly supervised by State, Tribal, or Federal veterinarians that are on-site at the time of testing.
 - BTB QAVs for cervid tuberculosis may collect blood from approved cervids.
 - Only approved laboratories that utilize standard operating procedures approved by NVSL may perform the DPP as official test for bovine tuberculosis in approved cervids.
 - Include a copy of the VS Form 6-22 including all the tube numbers and animal ID's may also be included with the VS 10-4 to provide the tube numbers and animal ID's.
- Sample testing
 - Valid serum samples will be tested using the DPP test following test kit instructions and internal NVSL standard operating procedures.
 - If the DPP primary test result obtained is above the optical density reader value cutoff point that has been established, the test will be called positive.
 - Results of the DPP primary test will be reported to the submitting accredited veterinarian, the respective State animal health official, Assistant District Director

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- (ADD), and district or designated epidemiologist of the State where the animals are located. The district or designated epidemiologist will report to the CHG cervid staff epidemiologist.
- The District Epidemiologist or CHG cervid staff epidemiologist will classify the animals.
 - Animals negative on the DPP primary test should be classified as negative.
 - Animals non-negative on the DPP primary test should be classified as suspect unless the District or CHG cervid staff epidemiologist determines that a reactor classification is warranted.
 - Animals classified as suspect by a DPP primary test may be retested with the DPP test with a new blood sample drawn no sooner than 30 days after the initial sample was obtained.
 - Animals testing negative on the second DPP test should be classified as negative.
 - Animals that are non-negative on two successive DPP tests should be classified as reactor.
 - Any exceptions to reactor classification must be justified by the District epidemiologist in writing and have the concurrence of the Cervid Health staff epidemiologist.
 - The animal is then handled according to its classification following TB program regulations.
 - TB Program Testing Protocol
 - If an animal tests non-negative to the primary DPP serological test, it must be retested in not less than 30 days using the DPP serological test as the secondary test.
 - If an animal tests non-negative to the SCT, it must be retested using the comparative cervical tuberculin skin test (CCT) as the secondary test.
 - The CCT will not be used as a primary test for any animals.
 - If parallel testing is performed with the DPP and SCT tests, it must be completed with permission from and consultation with the District epidemiologist and the CHG cervid staff epidemiologist. The testing protocol, timing of different tests, interpretation of the tests, classification of the animals, and disposition of the animals must be determined in the protocol before the testing. Secondary tests to any non-negative primary test must follow the first two instructions above.
 - In routine herd testing, different groups of animals within a herd may be tested using the different methods (i.e., bucks tested serologically and does tested via the skin test) if a different VS Form 6-22 is used for each group of animals. However, individual animals testing non-negative to a primary test must be followed up with a test of the same test method as required in the first two instructions above.
 - In affected herds or herds under investigation, a testing protocol using serological and skin tests separately or in series or parallel may be devised and used by the designated or District epidemiologist and CHG cervid staff epidemiologist in consultation.

8.4.6 Post-mortem tests for bovine tuberculosis

8.4.6.1 Test description

- A combination of histopathology, mycobacterial culture, PCR, and/or genotyping is used to further evaluate tissues collected during necropsy or at the time of slaughter for the presence of *M. bovis*.
 - **Histopathology** is a rapid method of identifying structural changes in tissues associated with mycobacterial infections. Mycobacteriosis compatible: This diagnosis means that the lesion is consistent with tuberculosis and the granuloma contains acid-fast bacteria. Because the species of acid-fast bacteria causing this lesion cannot be determined using histopathology alone, a diagnosis of mycobacteriosis compatible is not a diagnosis of infection with *M. bovis*.
 - **PCR**
 - May be performed after a mycobacteriosis compatible diagnosis on formalin fixed paraffin embedded (FFPE) tissue and is used to determine the presence of genetic material from the *Mycobacterium tuberculosis* complex (which includes *M. bovis*, *M. tuberculosis*, and several other species), *M. avium* and *M. a. paratuberculosis* in tissue.
 - May be performed on grossly lesioned fresh or borate tissue. PCR may be used in place of culture for routine slaughter surveillance samples. Program staff must approve the use of PCR in place of culture for all other cases. PCR is not an appropriate test for grossly normal tissue.
 - **Mycobacteriologic culture** isolates mycobacteria from the tissue and the permits definitive identification and genotyping of *M. bovis*.
 - **Genotyping** using whole genome sequencing is a high resolution method for determining the relatedness of isolates.

8.4.6.2 Appropriate use of the tests

- A combination of histopathology, mycobacterial culture, genotyping, and/or PCR testing is used as the final stage of testing for bovine tuberculosis and permits the final classification of suspect, reactor, and exposed animals as infected with *M. bovis* or negative.
- Animals that have acid fast bacteria recovered on culture that are speciated as *M. bovis* are confirmed tuberculosis-infected.
- Animals that have mycobacteriosis compatible lesions or are epidemiologically linked to *M. bovis* cases and are also PCR-positive for *Mycobacterium tuberculosis* complex will be classified as confirmed tuberculosis-infected.

8.4.6.3 Official testers

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- *Sample collection at slaughter of regular kill animals.* FSIS food inspectors and public health veterinarians may collect granulomatous lesions detected at the time of routine post-mortem slaughter inspection of regular-kill animals.
- *Sample collection at slaughter of bovine tuberculosis reactors, -suspects, or -exposed animals.* State, Tribal or Federal veterinarians may conduct post-mortem procedures and collect tissues from animals that are classified as bovine tuberculosis reactors, -suspects or -exposed.
- *Sample collection at the time of on-farm necropsy of bovine tuberculosis reactors, -suspects, or -exposed animals* State, Tribal or Federal veterinarians may conduct necropsies and collect tissues from animals that are classified as bovine tuberculosis reactors, -suspects or -exposed.
- Approved animal health technicians employed by a State, Tribal, or Federal government may collect tissues when approved to do so by such governments and directly supervised by State, Tribal, or Federal veterinarians.

8.4.6.4 *Sample collection at slaughter of regular kill animals*

Refer to [FSIS Directive 6240.1](#) for detailed instructions concerning the inspection and collection of tissue samples at the time of slaughter.

8.4.6.5 *Sample collection at slaughter: Bovine tuberculosis-reactors, -suspects, or -exposed animals*

State and federal animal health officials should complete the following actions when bovine tuberculosis-reactor, -suspect, or -exposed animals are to be presented for slaughter:

- Identify animals, complete VS Form 1-27, Permit for Movement of Restricted Animals, and seal truck, as described in Appendix D of the [National Veterinary Accreditation Program Reference Guide](#).
- Categorize bovine tuberculosis-exposed animals as either:

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- Category 1: Diagnostic exposed animals
 - These are animals that have been moved from a bovine tuberculosis-affected 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.
 - FSIS inspectors will perform a modified expanded post-mortem inspection procedure on these animals.
 - Category 2: Animals that are part of a known affected herd.
 - These are test negative or untested animals which may move to slaughter as regular culls or by entire herd.
 - FSIS inspectors will perform a regular post-mortem inspection procedure on these animals.
- The AD in the State or Tribe of origin should alert the AD in the State or Tribe where the animals are to be slaughtered of the pending shipment.
 - The AD in the State or Tribe where the animals are to be slaughtered should inform FSIS personnel at the receiving establishment prior to the animals' arrival.
 - VS or State/Tribal animal health personnel should be present to oversee and assist with the collection of tissue specimens as described in [FSIS Directive 6240.1](#).
 - Submit specimens collected from bovine tuberculosis-reactors, -suspects, or -exposed animals at slaughter to NVSL on a 10-4 form, noting if carcasses are retained.

8.4.6.6.1 Materials and equipment

Sampling and Shipping Supplies

The NVSL will provide the appropriate sampling and shipping supplies upon request. Requests can be emailed to NCAH.Shipping@aphis.usda.gov. The supplies needed to sample one animal include:

- For fixed tissues, at least 3 jars (90 mL) of 10% neutral buffered formalin.
- For fresh tissues, either at least 3 plastic sample bags per animal or at least three 4-ounce jars of saturated sodium borate solution.
- 8 barcodes: 3 for the buffered formalin jars (head and chest, abdomen), 3 for the plastic bags or sodium borate jars, 1 for the form and 1 red top tube for blood/serum (if applicable)
- FedEx shipping label
- Form 10-4
- Form 10-7

Necropsy Tools

Suggested necropsy supplies include:

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- Sharp knife (hunting knives and slaughterhouse boning knives work well)
- Scissors
- Rat-tooth forceps
- Cutting board
- Small and large shears (lopping shears or ratcheted rib cutters for rib cage/sternum)
- Scalpels (disposable scalpels are highly recommended)

Other Supplies

Other suggested supplies include:

- Disinfectant
- Scrub brush
- Large rubber tub (for disinfecting boots and necropsy tools)
- Datasheets
- Plastic bags (large for carcass disposal and small for sample collection)
- Sharpie® or indelible marker (for labeling)
- Pen (for filling out datasheets)
- Plastic sheets, wood chips, pet litter, or other absorbents (for floors in work area)
- Sharps container
- Biohazard waste bag
- Digital camera
- Ruler (for measuring lesions and/or tissue)

8.4.6.6.2 Personal Safety Guidelines and Equipment

Bovine tuberculosis is zoonotic and presents a risk to human health and safety. Because of this risk, all carcasses should be handled with caution and considered potentially infectious. Precautions for personal safety should be exercised.

Do not eat, drink, or smoke while dissecting a carcass or collecting samples. Establish a clean work zone and a contaminated work zone (clean/dirty line) with an area to disinfect supplies, equipment, and personnel between the two areas. Place datasheets, camera, and other non-disposable equipment in plastic bags or containers that can be disinfected or discarded.

All personnel conducting necropsies or handling animals that may be infected with bovine tuberculosis should have a bovine tuberculosis test prior to any potential exposure and annually thereafter (or as recommended by an occupational health professional).

Safety and Personal Protective Equipment (PPE)

Wearing protective gear will minimize the possibility of contact with infectious agents in body fluids and aerosols and reduce the risk of human infection. All necropsy tools and instruments should be disinfected before and between necropsies, and after sampling to prevent cross-contamination and infection.

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The following PPE are recommended during sample collection:

- Heavy-duty disposable gloves (rubber or nitrile)
- Cut-resistant mesh glove on non-dominant hand
- Goggles, safety glasses, or face shield
- Disposable apron or apron that can be disinfected
- Forearm protectors
- Cloth or Tyvek® coveralls
- Rubber boots
- Hair net or hat that can be disinfected
- Respirator (N95 mask at a minimum)

Work upwind of carcasses when performing necropsies outdoors. Always wash hands and exposed skin with soap and warm water or an alcohol based cleanser after collecting samples.

Handling Harmful Substances

Sodium borate and 10% buffered formalin are hazardous substances that can be inhaled or absorbed through the skin. Carefully handle all harmful substances when sampling and shipping.

Disinfectants are also potentially hazardous and should be handled with care. The Material Safety Data Sheets for each chemical should be reviewed prior to use to ensure that collectors are aware of the dangers associated with handling the disinfectants and chemicals and take the appropriate precautions.

Carcass Disposal and Disinfectants

The carcass and all tissues from the carcass should be disposed of according to State, Tribal and local animal carcass disposal regulations. Depending on the State, Tribe, or area, methods may include burial, incineration, composting or double-bagging and transporting to a landfill. All contaminated paper or plastic materials should be considered hazardous waste and should be thoroughly disinfected, incinerated or double-bagged and disposed of at the landfill (if permitted).

All blood and tissue should be removed from necropsy instruments and tools with soap and water, rinsed, and subsequently disinfected with an approved disinfectant for bovine tuberculosis between necropsies. If disinfectants are not used between animals, false positives may be identified.

Gloves also should be changed between animals. Necropsy boots, aprons and contaminated clothing should be cleaned and thoroughly disinfected upon completion of sample collections. External surfaces of containers with samples should be disinfected.

The products listed in the table below are effective, environmentally friendly disinfectants for use against bovine tuberculosis. Additional approved bovine tuberculosis disinfectants can be found in the U.S. Environmental Protection Agency Office of Pesticide Programs List B: EPA's Registered Tuberculocide Products Effective Against *Mycobacterium tuberculosis*.

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Disinfectant	Time to Effectively Disinfect	Environmentally Friendly	Manufacturer
Oxivir bovine tuberculosis	5 minutes	Active ingredients break down to water and oxygen	Johnson Diversey
Opti-Cide 3	3 minutes	Contains no dangerous phenols, chlorine, artificial dyes or perfumes	Micro-Scientific Industries
Clorox Bleach (Mix 1 part bleach with 9 parts water)	5 minutes	Product contains no free chlorine and breaks down into salt and water after use; does not contain dioxins or contaminate groundwater	Clorox Company

Disposal of Gloves and Sample-Related Waste

Spray or soak waste with disinfectant and place in bag. Then spray or soak bag with disinfectant, place in another bag, and dispose at the landfill (if permitted).

8.4.6.6.3 Collecting Specimens

General Recommendations

Despite the stringent decontamination protocols used in the laboratory, tissue specimens can still be overgrown by environmental fungi and bacteria, thereby impeding the ability to recover any viable mycobacteria present in the tissues. To minimize overgrowth, it is important to collect tissues from the animal as soon as possible post mortem. Collect tissues from animals within 2 hours whenever possible. When performing the necropsy, collect the tissues using aseptic techniques from the head, thoracic cavity and abdominal cavity (in that order) to minimize cross-contamination.

Samples to Collect

In summary, representative lymph nodes from the head, thorax, and abdomen as well as any lesions are collected in both formalin for histopathology and either in sodium borate jars or plastic bags for fresh chilled samples used for culture on all animals. Beginning with the head, the medial and lateral retropharyngeal lymph nodes, mandibular lymph nodes, and parotid lymph nodes are incised, examined, and subsampled for both histopathology and culture. The subsamples are placed in a formalin jar labeled “head” and a sodium borate/plastic bag for culture also labeled “head”. Next the thoracic lymph nodes including the tracheobronchial and mediastinal lymph nodes are incised, examined, and subsampled for both histopathology and culture. The subsamples are placed in a formalin jar labeled “thorax” and a sodium borate/plastic bag for culture also labeled “thorax”. The abdominal lymph nodes including the mesenteric and hepatic lymph nodes are incised, examined, and subsampled for both histopathology and culture.

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The subsamples are placed in a formalin jar labeled “abdomen” and a sodium borate/plastic bag for culture also labeled “abdomen”. If granulomatous lesions in any of these lymph nodes are identified, ideally they should be placed in a separate formalin jar and sodium borate/plastic bag labeled with the name of the lymph node and “lesion”. The following tissues are examined, but only subsampled and submitted to the laboratory if there are granulomatous lesions identified: Lung, pleura, liver, spleen, female reproductive organs, pre-scapular lymph nodes, cervical lymph nodes, popliteal lymph nodes, mammary lymph nodes, iliac lymph nodes.

All animal IDs should be collected with at least a dime size piece of tissue left associated with the identification devices. For backtags, removing the tag by pulling off the hide allowing the hair roots to remain attached to the tags is desirable.

Blood for serum should also be collected from animal exhibiting gross lesions suggestive of bovine tuberculosis.

Additional guidance on subsampling of tissue and tissue preservation

1. Always clean and disinfect instruments between necropsies of each animal. If disposable scalpels are available, discard them after each animal. Thoroughly rinse instruments after disinfecting to ensure that the samples are not inadvertently disinfected. Change gloves between the necropsy of each animal.
2. Remove excess fat from tissue samples.
3. If there are lesions identified which are too small to subdivide for both histopathology and culture, submit the small lesion only for histopathology.

Tissue preservation

Tissue Preservation Method	Test Type	Situation
Formalin	Histopathology	All instances
Sodium borate	Culture	Preferred if 10 or fewer animals are sampled
Refrigerated or Frozen in leak-proof bags	Culture	For large number of samples or for low priority research.

- Samples submitted for histopathology should be placed in 10% buffered formalin jars. Use a 10:1 formalin to tissue ratio when submitting samples for histopathology. Tissues placed in formalin should be bread sliced so that they are approximately 1 cm or less thick (e.g., the width of a pencil).
- For bacteriologic examination -- cut tissues approximately 2 to 5 cm thick and place in a sodium borate solution at a 1:1 tissue to preservative ratio.

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- Do not make additional incisions into the sample because sodium borate is bacteriocidal.
- Sodium borate solution is a supersaturated solution. It is normal to see crystals in the bottles containing the solution.

Sodium borate is generally preferred over fresh/frozen when submitting less than 10 animals for culture. Use equal parts of tissue and sodium borate (if appropriate) when submitting for mycobacteriologic culture. An advantage of sodium borate is that the tissue can be preserved without refrigeration, and it decreases the risk of tissue contamination by other bacteria. However, if the sample does not arrive at the lab for culture testing within 72 hours, the sodium borate may penetrate the tissue sample and kill any mycobacteria that may be present, increasing the risk of a false negative culture result.

For large culture submissions and lower priority research or surveillance projects, samples submitted for culture should be placed in leak proof plastic bags. Refrigerate or place in a cooler with cold packs until shipped to the laboratory. If fresh tissues for culture will be held more than 72 hours prior to shipping, freeze at -20°C and ship the tissues frozen on ice packs. Note do not freeze formalin fixed tissues.

- Tighten the caps on specimen containers and seal with Parafilm®. Electrical tape also can be used if Parafilm® is not available.
- After sample collection, disinfect the outside of each plastic bag or container in an approved disinfectant for bovine tuberculosis. Use caution to ensure that no disinfectant enters the plastic bag or the sodium borate container. Remember to keep the surface wet with disinfectant solution for the required contact time (see the previous table or refer to the product label). Rinse with water after the contact time requirement has been met.

8.4.6.6.4. Completion of submission forms

- [Forms and detailed instructions](#) are available for download.
- For samples submitted from routine postmortem examination at slaughter
 - Submit VS Form 6-35.
- For all bovine tuberculosis-reactor, -suspect, or -exposed animals
 - Submit VS Form 10-4 AND the supplemental tuberculosis collection form VS 10-7.
 - Provide all available information (owner, material submitted, animal identification, examination requested).
 - A 10-4 form must accompany each shipment and can include multiple animals.
 - Fill out a separate supplemental 10-7 for each individual animal.
 - An accurate description of the lesions is very important (size, color, location, and consistency) as is information on the type of carcass (species, sex, age, condition).
 - Be certain the animal identification on the forms correlates with the animal identification on the sample containers.

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8.4.6.6.5 *Packaging samples for shipment to NVS*

- Send all of tissues for each animal in a single shipping container overnight.
- Seal a copy of the completed VS Form 10-4 and the supplemental VS Form 10-7 form in a plastic bag and place them between the Styrofoam™ cooler and the cardboard box. Do not place forms inside the Styrofoam™ cooler.

8.4.6.6.6 *Communication when shipping samples to NVSL*

Contact the laboratory if planning on submitting a large number of animals (>10) or with any questions. The laboratory may be contacted by email at NvslCaseCoordinator@aphis.usda.gov or by phone:

- Histopathology: (515) 337-7521
- Mycobacteriology: (515) 337-7388
- Serology: (515) 337-7565

8.4.6.6.7 *Specimens from reactor swine*

Tissue samples from swine that are tested and classified as reactors as part of an investigation should be submitted when deemed necessary.

8.4.6.6.8 *Specimens from other species*

- It is the policy of VS to provide diagnostic assistance for other species of animals (from zoos, animal compounds, primate centers, roadside parks, etc.) when deemed necessary by the AD. For guidance regarding on sampling of other species, contact the appropriate bovine tuberculosis epidemiologist designated by the District Director or CHG specialist.
- NVSL will charge a user fee for avian, dogs, cats, nonhuman primates, reptiles, amphibians, environmental samples, and elephant trunk washes.
- No charge will be made for other zoo mammals when *M. bovis* is suspected.
- If submitters need to open an NVSL account, they can call 515-663-7571, User Fee Help Line.

8.4.6.6.9 *Specimens from special studies*

- Specimens from special studies must be approved in advance by NVSL and commodity center staff.
- Contact the appropriate bovine tuberculosis epidemiologist designated by the District Director or commodity center specialist for guidance regarding the approval process.

8.4.7 Supplemental tests for bovine tuberculosis

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8.4.7.1. *Tissue matching (microsatellite genotyping)*

- Appropriate use of the test
 - Tissue matching (animal identification devices with the formalin fixed tissue) will be conducted on all slaughter trace (6-35) cases with IDs submitted. It will also be done on all culture, histology discrepant cases.

8.4.7.2. *Blood collection for the bovine tuberculosis serum bank*

- It is preferred that serum samples be collected from live animals sent for necropsy that are likely to be infected with bovine tuberculosis. Such animals include suspect or reactor animals from known bovine tuberculosis-infected herds, tracebacks from a known bovine tuberculosis-infected herd, or other animals as suggested by an epidemiologist designated by the District Director or a commodity health group specialist.
- If it is not feasible to collect blood from an animal prior to necropsy and bovine tuberculosis or tuberculosis-like granulomas or lesions are observed in the carcass, use a needle and syringe to extract as much blood as possible from the heart.
- The following is a suggested sampling protocol if serum samples are collected and submitted to the bovine tuberculosis serum bank at NVSL:
 - Place extracted blood into red-top or serum-separator tubes (collect at least three 10ml tubes, if possible, so at minimum 10mls of serum can be harvested from the blood tubes).
 - Blood should be left undisturbed for approximately 30 minutes at room temperature to encourage clot formation prior to centrifugation.
 - Centrifuge 10 minutes to separate the serum from the blood cells. (NOTE: If it is blood collected from the heart, centrifuge for 10 minutes at a minimum of 1,800 revolutions per minute to separate the serum from the blood cells. If a centrifuge is not available, serum can be obtained by placing the blood tube in a refrigerator overnight to allow the clot to form and contract.
 - Decant or transfer the serum into a new red top tube or other vial that has a screw top lid. If multiple tubes of blood have been collected, it is satisfactory to combine harvested serum into one tube (or more, if needed).
 - Identify the serum tubes in a fashion so that can be easily correlated to the animals listed on a lab submission form or a copy of the tuberculosis test chart.
 - It is preferred to maintain serum at refrigerated temperatures until the sample is shipped to NVSL.
 - Refrigerated serum should be shipped to NVSL with ice packs within 2 weeks of collection.
 - Submit, at minimum, 1 mL of serum (more if possible) to the bovine tuberculosis serum bank from each animal.
 - If it is more convenient for a facility to submit frozen serum to the bovine tuberculosis serum bank, transfer 0.5 mL of serum into as many 1.2 mL polypropylene Cryovials® that will contain the harvested serum. Identify the tubes to be submitted in a fashion that can be easily correlated to animals listed on a lab

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- submission form or a copy of the tuberculosis test chart. Freeze the Cryovials® at -20°C.
- Frozen serum can be shipped to NVSL once a month on dry ice to ensure the samples remain frozen during shipping. This will reduce the number of freeze-thaw cycles during processing at NVSL to ensure that sample quality is maintained.
 - For all samples submitted to the bovine tuberculosis serum bank at NVSL, provide skin test and any other pre-necropsy bovine tuberculosis testing information (gamma interferon or other serology tests) to the serum bank coordinator in order to characterize the serum samples.

Appendix 1: Animal Health Plan Template

- APHIS will evaluate a State's or Tribe's animal health plan using the criteria listed below. APHIS will approve animal health plans that sufficiently address all of the categories of information.

Category 1: Laws and Regulations

I. Submission requirements

- A. A statement confirming that the State or Tribe has a legal and regulatory basis for the activities and measures specified within the animal health plan.
- B. A citation of the laws or regulations that provide the specific authority to:
 1. List brucellosis and bovine tuberculosis as reportable diseases.
 2. Quarantine animals, herds of animals, and/or geographic areas.
 3. Require animal owners to present their animals for program purposes.
- C. A list of any specific organizations or entities within the State or Tribe to which authority for the control of brucellosis and/or bovine tuberculosis has been delegated.
- D. Identification of the organization or entity (i.e., wildlife or agriculture) within the State or Tribe that has the authority to control disease in captive cervids.
- E. A summary of amendments to State or Tribal regulations necessary to implement and enforce the animal health plan.
 1. Note whether previous Federal brucellosis or tuberculosis regulations or policies (i.e., Program Standards) are incorporated into State or Tribal regulations.
 2. An outline of the expected timeline to enact the proposed amendments.
 3. Please note: Once State or Tribal regulatory amendments become effective, amendments must be made to the animal health plan to describe the updated laws and regulations. (See [Amendments to animal health plans.](#))

II. Evaluation criteria

- A. All requested information was submitted.
- B. The lists, citations, and summaries show that the State or Tribal animal health and wildlife authorities can implement and enforce the animal health plan.

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- C. The lists show that the State or Tribe has the authority to:
 - 1. List brucellosis and bovine tuberculosis as reportable diseases.
 - 2. Quarantine animals, herds of animals, and/or geographic areas.
 - 3. Require animal owners to present their animals for program purposes.
- D. If necessary, the State or Tribe has developed a plan to amend its regulations to meet applicable requirements of the brucellosis and bovine tuberculosis programs. (See Approval of animal health plans.)

Category 2: Organization and Infrastructure

I. Submission requirements

- A. A description of the organization and infrastructure of the animal health and wildlife authorities within the State or Tribe.
 - 1. A description of the animal health and wildlife workforce within the State or Tribe that is available to implement and perform activities or maintain and enforce measures specified within the animal health plan, including:
 - a. The number of Category II federally accredited veterinarians and the number of qualified accredited veterinarians (BTB QAVs) for bovine tuberculosis.
 - b. The number of State, Tribal, and Federal veterinarians and animal health technicians.
 - c. The number of State, Tribal, and Federal wildlife biologists and veterinarians.
 - d. The name of the epidemiologist(s) designated by the District Director for both brucellosis and bovine tuberculosis in the State or Tribe.
 - e. The diagnostic laboratory capability within the State or Tribe.
 - 2. A description of any formal or informal agreements that allow access to additional resources from other States, Tribes, or the Federal government, including:
 - a. Any agreements with other States, Tribes, or Federal entities that allow access to additional personnel or allow for a coordinated, joint response to any of the information categories in the animal health plan.
 - b. Any agreements with other States, Tribes, or Federal entities to provide laboratory services (including fee-for-service agreements).

II. Evaluation criteria

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- A. All requested information was submitted.
- B. The descriptions and lists show that the State or Tribe has sufficient resources to implement, maintain, and enforce its animal health plan.
- C. The State or Tribe has identified epidemiologist(s) designated by the District Director for brucellosis and bovine tuberculosis.
 - 1. States or Tribes may identify the same individual to serve as the epidemiologist designated by the District Director for both brucellosis and bovine tuberculosis.

Category 3: Responsible persons

I. Submission requirements

- A. List the name and contact information for the responsible person that the State or Tribe has designated to oversee implementation, performance, and enforcement of animal health activities and measures carried out under the plan within the State or Tribe.
- B. List the name and contact information for the person that the State or Tribe has designated to oversee implementation, performance, and enforcement of wildlife activities and measures carried out under the plan within the State or Tribe.

II. Evaluation criteria

- A. All requested information was submitted.
- B. The State or Tribe has identified the responsible person designated to oversee implementation, performance, and enforcement of the animal health activities and measures under the plan within the State or Tribe.
 - 1. This individual will typically be the State or Tribal Veterinarian or his or her designee.
 - 2. States or Tribes may identify the same individual to serve as the responsible person for the wildlife activities and measures carried out under the plan.
- C. The State or Tribe has identified the responsible person designated to oversee implementation, performance, and enforcement of the wildlife activities and measures carried out under the animal health plan within the State or Tribe.
 - 1. States or Tribes may identify the same individual to serve as the responsible person(s) for the animal health activities and measures carried out under the plan.

Category 4: Program animal demographics

I. Submission requirements

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- A. A description of the program animal demographics within the State or Tribal lands, including:
 - 1. The approximate number of cattle, bison, and captive cervid herds by species, and the approximate number of animals within these herds.
 - 2. The types of cattle, bison, and captive cervid herds.
 - a. For example, in cattle, herds should be identified as beef, dairy, or mixed herds.
 - b. In captive cervids, use should be identified if possible (e.g., a shooter herd, a breeding herd, other).
 - 3. A brief description of the geographic distribution of these herds (optional).
- B. A description of the approximate number and geographic distribution of any animal concentration points within the State or Tribal lands. Examples of animal concentration points include sale barns, buying stations, slaughtering establishments, and feedyards.
- C. A brief description of the general movement patterns of program animals within the State or Tribal lands (optional).

II. Evaluation criteria

- A. All requested information was submitted.
- B. The descriptions show that the State or Tribe has an understanding of the numbers and distribution of program animals within the State or Tribal lands and their impact on disease surveillance, management, and response activities and measures, as described in their animal health plan.
- C. The State or Tribe provides updates concerning this category of information in their annual report.

Category 5: Surveillance

I. Submission requirements

- A. A statement verifying that the State or Tribe agrees to participate in the National Surveillance Plans for brucellosis and bovine tuberculosis or a description of an alternate plan proposed by the State or Tribe.
- B. A description of the core and refresher training provided to BTB QAVs in the State or Tribe including the elements covered in the training and the approach used to verify that veterinarians can demonstrate specific skills.
- C. A description of how performance of BTB QAVs and regulatory veterinarians is monitored in the State or Tribe including the actions taken when a BTB QAV or regulatory veterinarian fails to meet the performance standards.

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- D. A description of the approach for conducting targeted surveillance in known source population(s) for brucellosis and/or bovine tuberculosis in wildlife, when identified. The description should include the goal for detection level, sample size goal, and sample selection process.
- E. A description of the approach for conducting targeted surveillance for brucellosis and/or bovine tuberculosis in at-risk population(s) of program animals, when identified. The description should include the goal for detection level, sample size goal, and sample selection process in the description.

II. Evaluation criteria

- A. All requested information was submitted.
- B. The State or Tribe has agreed to participate in the National Surveillance Plans for brucellosis and bovine tuberculosis, or the State or Tribe has submitted an alternate plan that meets or exceeds the level of disease detection for the National Surveillance Plans and is approved by APHIS.
- C. The State or Tribe has described an approach for training BTB QAVs that includes all necessary elements and verifies that veterinarians can demonstrate specific skills.
- D. The State or Tribe has described an approach for monitoring BTB QAV and regulatory veterinarian performance and enforcing compliance with the performance standards.
- E. States or Tribes that identified known source population(s) for brucellosis and/or bovine tuberculosis have described an approach for targeted surveillance sufficient to detect infection at the specified level within the source population.
- F. States or Tribes that identified population(s) at-risk for transmission of brucellosis and/or bovine tuberculosis have described an approach for targeted surveillance that meets or exceeds the requirements of § 76.6.

Category 6: Sources of brucellosis and bovine tuberculosis

I. Submission requirements

- A. A description of the known sources of brucellosis or bovine tuberculosis that pose a risk of disease introduction into program animals within the State or Tribal lands, including:
 - 1. The approximate number of herds or wildlife populations and animals in these herds or population(s) within the State or Tribal lands that are known sources of brucellosis or bovine tuberculosis.
 - 2. The approximate prevalence of brucellosis or bovine tuberculosis infection in those population(s).
 - 3. The geographic distribution of the population(s) within the State or Tribal lands.

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4. A description of any other factors that make the population(s) a potential source of brucellosis or bovine tuberculosis spread to program animals within the State or Tribal lands.
- B. An assessment of the likelihood of transmission of brucellosis or bovine tuberculosis from these sources to program animals within the State or Tribal lands, including a description of:
1. The potential for exposure of program animals within the State or Tribe to these known source populations.
 2. Factors, other than mitigation measures that are or would be implemented by the State or Tribe, that may influence this potential for exposure (i.e., the movement patterns of animals, seasonal variations in exposures, etc.).
 3. A summary of or citation to key findings from any formal risk assessments concerning the risk of spread of brucellosis or bovine tuberculosis within the State or Tribe, if available.
- C. If sources of brucellosis or bovine tuberculosis that pose a risk of disease introduction into program animals are not identified within the State or Tribe, a justification should be provided describing the information considered and the process used to reach this conclusion.

II. Evaluation criteria

- A. All requested information was submitted.
- B. The descriptions show that the State or Tribe has considered sources of brucellosis or bovine tuberculosis that may pose a risk of transmission to program animals in the State or Tribal lands.
- C. The descriptions show that the State or Tribe has conducted an assessment of the likelihood of transmission of brucellosis or bovine tuberculosis from these sources to program animals within the State or Tribal lands.

Category 7: Risk mitigation measures

I. Submission requirements

If the State or Tribe has identified known source populations of brucellosis or bovine tuberculosis that pose a risk of disease introduction into program animals within the State or Tribal lands, a description of the measures that the State or Tribe has implemented or would implement including a timeline for implementation to prevent and/or mitigate the risk that program animals within the State or Tribal lands will become infected with brucellosis or bovine tuberculosis.

- A. For wildlife sources, examples of mitigations may include, but are not limited to:

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1. Fencing to limit wildlife/livestock exposures.
2. Vaccination of wildlife.
3. Animal density reduction.

B. For livestock sources, examples of mitigations may include, but are not limited to:

1. Affected herd management plans.
2. Use of calfhood vaccinations for brucellosis.
3. Pre- and post-movement testing requirements.

II. Evaluation criteria

1. If necessary, all of the requested information was submitted.
2. Descriptions reflect that prevention and/or risk mitigation measures are or will be in place to reduce disease spread within the State or Tribe.

Category 8: Disease investigation, management, and response

I. Submission requirements

- A. A statement verifying that the State or Tribe agrees to meet the minimum requirements for epidemiological investigation and affected herd management as set forth in 9 CFR 76.7.
- B. A description of any epidemiological investigation and affected herd management activities that the State or Tribe plans to take in response to occurrences of brucellosis and/or bovine tuberculosis within program animals in the State or Tribal lands that differ from or exceed the minimum requirements set forth in 9 CFR 76.7.

II. Evaluation criteria

- A. All requested information was submitted.
- B. The State or Tribe has agreed to meet the minimum requirements for epidemiological investigation and affected herd management as set forth in 9 CFR 76.7; OR
- C. The State or Tribe has described any planned epidemiological investigation and affected herd management activities that differ from or exceed the minimum requirements as set forth in 9 CFR 76.7.

1.1.2 Proposal to recognize a management area as part of the animal health plan

States and Tribes requesting APHIS recognition of a management area will include an Appendix 8: Amendment to Animal Health Plan for Recognition of a Management Area that contains the categories of information required in 9 CFR 76.5.

- The information in the animal health plan will address activities or measures throughout the State or Tribal lands, while the information in this optional section will address those activities or measures conducted within the proposed management area.

1.1.3 Joint responses to certain information categories in animal health plans

- States and Tribes with limited infrastructure and resources to implement the animal health plans, small numbers of program animals, or other circumstances may elect to coordinate surveillance, risk mitigation, and management and response activities or measures across multiple States, Tribal lands, or both.
- Each State or Tribe must submit a separate animal health plan. However, a joint response addressing coordinated activities that are similar for all participating States and Tribes may be submitted for the following categories:
 - Category 5: Surveillance.
 - Category 6: Sources of brucellosis and bovine tuberculosis.
 - Category 7: Risk mitigation measures.
 - Category 8: Disease investigation, management, and response.

1.1.4 Submission of animal health plans

While APHIS prefers States and Tribes to submit animal health plans electronically to the Associate District Director (ADD), they can submit a paper copy to the [AD](#).

1.1.5 Approval of animal health plans

The steps involved in internal review of animal health plans are listed below. APHIS' notice-based process for approving an animal health plan is shown in Figure 1.

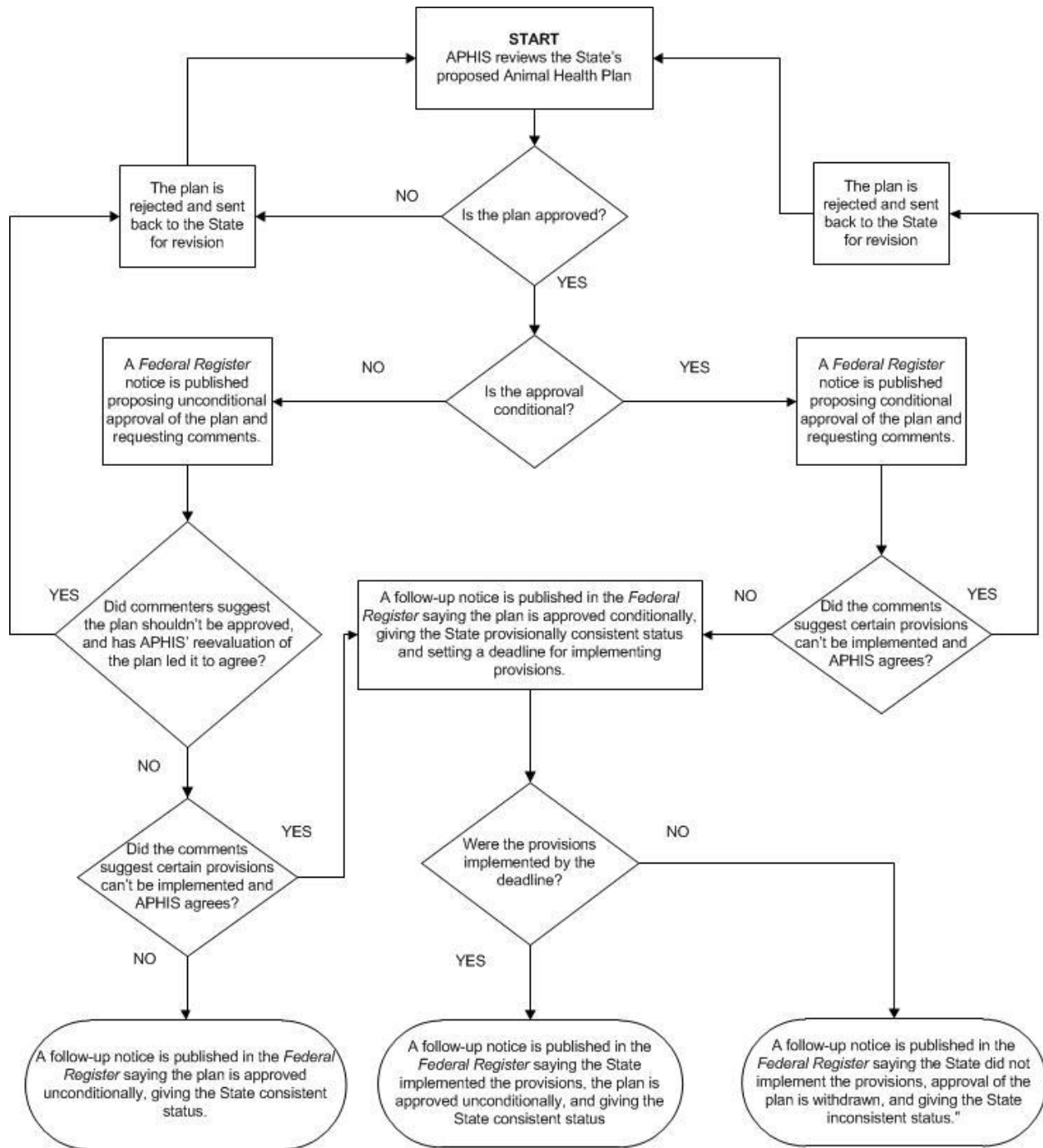
- Upon receipt, the AD will review the proposed animal health plan to ensure that the plan is complete and that the submitted information is accurate. The AD will also verify that the State or Tribe has the ability to fully implement the plan.
- The VS District Director or designee will review the proposed animal health plan to ensure that the proposed activities meet the Federal regulations, policies, and performance standards for the brucellosis and/or bovine tuberculosis programs. The District Director may ask the State or Tribe to revise its animal health plan to correct inaccuracies, provide additional information, or address other issues before district approval.

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- After district approval, the Director of the VS CHG or designee will review the proposed animal health plan to verify that the State or Tribe meets the criteria for consistent or provisionally consistent status.
 - **Consistent status:** APHIS may propose to approve a plan “as-is” (unconditionally) when the contents of the plan meet all requirements and all components of the plan can be implemented immediately upon approval.
 - **Provisionally consistent status:** Alternatively, APHIS will propose to approve the plan conditionally when the State or Tribe has indicated that it cannot implement specific components of the plan immediately upon approval of the plan. APHIS will define a time period in which the outstanding components must be implemented.
 - **NOTE:** APHIS will propose to conditionally approve an animal health plan if a State or Tribe indicates in the plan that amendments to State or Tribal regulations are needed in order to fully implement the plan. APHIS will propose to initially classify the State or Tribe as provisionally consistent. APHIS will reclassify the State or Tribe as consistent if the amendments are enacted. APHIS may re-designate a State or Tribe as inconsistent if it is unable to effectively respond to or manage incidents of disease because it lacks the necessary authorities or if the amendments are not enacted within the specified time.
- Once APHIS determines to propose to approve the plan, APHIS will publish a notice in the *Federal Register* proposing to approve the plan and making it available for public review and comment.
- After the comment period ends, APHIS will review public comments and determine if the plan should be approved, either unconditionally or with conditions. State or Tribe status for brucellosis and/or bovine tuberculosis will be designated based on the type of approval of the animal health plan.
- If, based on the comments received, APHIS determines to approve the plan, the CHG Director will initiate publication of a subsequent notice in the *Federal Register* announcing the approval of the plan and designating the State or Tribe to be consistent or provisionally consistent for brucellosis and bovine tuberculosis.

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Figure 1 APHIS approval of animal health plans and initial designation of State and Tribe status for brucellosis and bovine tuberculosis



1.1.6 Amendments to animal health plans

- States and Tribes need to submit amendments to the animal health plan to:
 - Update the information contained within the approved plan to ensure that it is up-to-date and that the activities and measures described correspond to the risk of spread of brucellosis or bovine tuberculosis.
 - Address issues that led to redesignation to inconsistent status, if seeking reevaluation and redesignation back to consistent status.
 - Ask APHIS to recognize a management area. States and Tribes should amend their animal health plan to include information required in 9 CFR 76.5, Recognized management areas.
 - Show substantive changes in activities and measures related to the brucellosis and bovine tuberculosis programs within the State or Tribe.
- States should submit amendments for changes to the following categories prior to the implementation of the activities and measures:
 - Category 1: Laws and Regulations
 - Category 5: Surveillance
 - Category 6: Sources of brucellosis and bovine tuberculosis
 - Category 7: Risk mitigation measures
 - Category 8: Disease investigation, management, and response
- States will review their animal health plans as part of their annual report and submit amendments for changes to the other categories as needed.
- APHIS will ask States and Tribes to amend their animal health plan if we determine that the activities and measures in the plan no longer correspond to the risk of spread of brucellosis or bovine tuberculosis.
- Amendments to animal health plans are submitted and approved using similar internal review and public notification procedures as previously described for animal health plans (see [1.1.4 Submission of animal health plans](#))

1.1.7 Making animal health plans publicly available

States and Tribes should be aware that animal health plans and amendments to animal health plans will be publicly available. APHIS will publish a notice in the *Federal Register* proposing approval of an animal health plan and making the plan available for public review and comment through the www.regulations.gov Web site. APHIS may also post approved animal health plans on its Web site.

1.2 State and Tribal classifications

1.2.1 Initial designation of status

- APHIS will determine the initial classification of a State or Tribe based on the approval of an animal health plan as previously described (Figure 1).
- If the proposed rule supported by these Program Standards is finalized, States and Tribes should submit an animal health plan to APHIS within 6 months of the date of publication of that final rule in the *Federal Register*. Late submissions may result in an initial designation as an inconsistent State or Tribe.
- APHIS will designate a brucellosis program status and a bovine tuberculosis program status for each State and Tribe at a date to be announced through a notice in the *Federal Register*. See [Appendix 2: Statuses Applied to States and Tribes](#)

1.2.2 Tribal classifications

- Tribes may elect to submit an animal health plan and have a status designated for brucellosis and/or bovine tuberculosis that is separate from that of the State(s) in which the Tribal lands are located. The submission requirements and approval process for the animal health plan and the status designation process for States will also apply for Tribes.
- Otherwise, a Tribe's classification status will default to that of the State(s) in which the Tribal lands are located. When this occurs, States and Tribes are encouraged to develop the animal health plan collaboratively to ensure that surveillance, risk mitigation, and management and response activities and measures are coordinated.

1.2.3 Redesignation to lower status

- 9 CFR 76.3(c)(1), (c)(2), and (c)(3) list those conditions that may result in the redesignation of a State or Tribe to a lower status. Table 1 summarizes these conditions.
- Variances from the provisions of an approved animal health plan occur when a State or Tribe fails to implement or inadequately implements activities and measures specified in the plan that are within their direct control.
 - APHIS considers both the degree of variance from activities and measures specified in the animal health plan and the risk of disease spread posed by the variance when determining the status for a redesignation.
 - APHIS will consider an immediate redesignation to inconsistent status when a State or Tribe fails to implement or inadequately implements the surveillance, risk mitigation, and management and response activities or measures as described in the State's or Tribe's approved animal health plan.

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- APHIS will not re-designate States or Tribes that exceed the activities specified within their approved animal health plan to a lower status because of this excess.
- Failure to submit a required report occurs when a State or Tribe does not submit the required report within the specified time or submits an incomplete report and declines to provide additional information to complete the report as requested by APHIS.

1.2.4 Additional requirements for provisionally consistent States and Tribes

- APHIS will require States and Tribes that are re-designated to provisionally consistent status to take remedial measures to address the issue(s) that resulted in the redesignation. Failure to implement the remedial measures will result in the redesignation of the State or Tribe as inconsistent.
- APHIS will determine remedial measures on a case-by-case basis and may include:
 - Supplemental surveillance in program animals and/or wildlife.
 - Additional reporting requirements, such as an increased frequency of reporting summary information and progress reports concerning epidemiological investigations.
 - Amendments to the animal health plan to include the addition of specific surveillance, risk mitigation, and management and response activities or measures that must continue on an ongoing, long-term basis to reduce the risk of disease spread.
- APHIS may also impose additional restrictions on the interstate movement of program animals from provisionally consistent States and Tribes. (See Interstate Movement .)
- APHIS may require a compliance review when the State or Tribe is provisionally consistent (see [Program compliance](#) reviews.)

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Table 1 Summary of circumstances that may result in the redesignation of a State or Tribe to a lower status

	Consistent to Provisionally Consistent	Consistent to Inconsistent	Provisionally Consistent to Inconsistent
Animal health plan (§ 76.2)	Not applicable	The State or Tribe fails to amend its animal health plan in response to a request by APHIS to do so, and APHIS determines that this failure has resulted or may result in the spread of brucellosis or bovine tuberculosis.	The State or Tribe fails to amend its animal health plan in response to a request by APHIS to do so, and APHIS determines that this failure has resulted or may result in the spread of brucellosis or bovine tuberculosis.
Variance from the approved animal health plan	The State or Tribe fails to implement or perform an activity or maintain a measure within its animal health plan, and APHIS has determined that this failure may result in the spread of brucellosis or bovine tuberculosis.	The State or Tribe fails to implement or perform an activity or maintain a measure within its animal health plan, and APHIS has determined that this failure has resulted or may result in the spread of brucellosis or bovine tuberculosis.	The State or Tribe fails to implement provisions of its animal health plan within the time specified by APHIS or fails to take required remedial measures. The State or Tribe fails to implement or perform an activity or maintain a measure within its animal health plan, and APHIS has determined that this failure has resulted or may result in the spread of brucellosis or bovine tuberculosis.

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	Consistent to Provisionally Consistent	Consistent to Inconsistent	Provisionally Consistent to Inconsistent
Recognized management areas (§ 76.5)	Not applicable	APHIS has terminated recognition of the State’s or Tribe’s management area.	APHIS has terminated recognition of the State’s or Tribe’s management area.
National surveillance (§ 76.6)	The State or Tribe fails to meet national surveillance levels as specified within the National Surveillance Plan for brucellosis or bovine tuberculosis (or an alternative State or Tribal plan that has been approved by APHIS).	The State or Tribe refuses to participate in or otherwise conduct national surveillance as specified within the National Surveillance Plan for brucellosis or bovine tuberculosis (and does not have an alternative State or Tribal plan that has been approved by APHIS).	The State or Tribe fails to take required remedial measures regarding surveillance. The State or Tribe refuses to participate in or otherwise conduct national surveillance as specified within the National Surveillance Plan for brucellosis or bovine tuberculosis (and does not have an alternative State or Tribal plan that has been approved by APHIS).
Targeted surveillance in source populations (§ 76.6(b)(1))	The State or Tribe fails to conduct targeted surveillance of wildlife source populations.	Not applicable	The State or Tribe fails to take required remedial measures regarding targeted surveillance.
Targeted ongoing surveillance in at-risk populations (§ 76.6(b)(2))	The State or Tribe fails to conduct targeted surveillance of at-risk populations of program animals.	Not applicable	The State or Tribe fails to take required remedial measures regarding targeted surveillance.

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<p>Epidemiological Investigations (§ 76.7)</p>	<p>The State or Tribe fails to conduct an investigation of a program animal with non-negative test results for brucellosis, or fails to submit a report of this investigation.</p>	<p>On more than one occasion, the State or Tribe has failed to conduct an investigation of a program animal with non-negative test results for brucellosis, or to submit a report of this investigation.</p> <p>The State or Tribe fails to conduct epidemiological investigations of infected animals or affected herds as specified within the regulations.</p>	<p>The State or Tribe fails to take required remedial measures regarding investigations.</p> <p>On more than one occasion, the State or Tribe has failed to conduct an investigation of a program animal with non-negative test results for brucellosis, or has failed to submit a report of this investigation.</p> <p>The State or Tribe fails to conduct epidemiological investigations of infected animals or affected herds as specified within the regulations.</p>
<p>Affected Herd Management (§ 76.7(e))</p>	<p>Not applicable</p>	<p>The State or Tribe fails to conduct affected herd management as specified within the regulations.</p>	<p>The State or Tribe fails to take required remedial measures regarding trace investigations.</p> <p>The State or Tribe fails to conduct affected herd management as specified within the regulations.</p>
<p>Annual report submission requirements (§ 76.4(a))</p>	<p>The State or Tribe fails to submit an annual report on time.</p>	<p>On more than one occasion, the State or Tribe has failed to submit an annual report on time.</p>	<p>On more than one occasion, the State or Tribe has failed to submit an annual report on time.</p>

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<p>Initial epidemiological investigation situation report (§ 76.4(c))</p>	<p>The State or Tribe fails to submit an initial epidemiological incident report within 14 days of the submission deadline (i.e., within 29 days of the date when the State or Tribe has been notified that a sample from a program animal has a non-negative test result for brucellosis or <i>M. bovis</i> has been identified by an official testing laboratory).</p>	<p>On more than one occasion, the State or Tribe has failed to submit an initial epidemiological incident report within 14 days of the submission deadline (i.e., within 29 days of the date when the State has been notified that a sample from a program animal has a non-negative test result for brucellosis or <i>M. bovis</i> has been identified by an official testing laboratory).</p>	<p>On more than one occasion, the State or Tribe has failed to submit an initial epidemiological incident report within 14 days of the submission deadline (i.e., within 29 days of the date when the State has been notified that a sample from a program animal has a non-negative test result for brucellosis or <i>M. bovis</i> has been identified by an official testing laboratory).</p>
<p>Updated epidemiological investigation situation report (§ 76.4(d))</p>	<p>The State or Tribe fails to submit an updated epidemiological investigation situation report at least every 4 weeks following submission of an initial report, or more frequently as requested by the Administrator.</p>	<p>On more than one occasion, the State or Tribe has failed to submit an updated epidemiological investigation situation report at least every 4 weeks following submission of an initial report, or more frequently as requested by the Administrator.</p>	<p>On more than one occasion, the State or Tribe has failed to submit an updated epidemiological investigation situation report at least every 4 weeks following submission of an initial report, or more frequently as requested by the Administrator.</p>
<p>Closing report (§ 76.4(e))</p>	<p>On more than one occasion, the State or Tribe has failed to submit a closing report within 60 days following the conclusion of an epidemiological investigation.</p>	<p>On more than one occasion, the State or Tribe has failed to submit a closing report within 60 days following the conclusion of an epidemiological investigation, and APHIS has determined that this failure has resulted or may result in the spread of brucellosis or bovine tuberculosis.</p>	<p>On more than one occasion, the State or Tribe has failed to submit a closing report within 60 days following the conclusion of an epidemiological investigation, and APHIS has determined that this failure has resulted or may result in the spread of brucellosis or bovine tuberculosis.</p>

1.2.5 Reestablishment of consistent status

- An inconsistent State or Tribe must address the issue that led to redesignation and submit an animal health plan or amendments to an existing plan for review and approval, as previously described (see Figure 1). Additionally, States and Tribes must submit any outstanding reports before APHIS will consider redesignating the State or Tribe to consistent status.
- APHIS will redesignate a provisionally consistent State or Tribe as consistent if the State or Tribe completes remedial measures required by APHIS.

1.2.6 Notification of redesignation

- APHIS will publish a notice in the *Federal Register* to announce all status changes.
- For all changes, the notice will give the status redesignation and state the reason(s) that led to the redesignation.
- The *Federal Register* notice that announces the redesignation of a State or Tribe from consistent to provisionally consistent status will also describe remedial measures that APHIS considers necessary for the State or Tribe to complete to regain consistent status. It may also specify restrictions on the interstate movement of program animals or other program requirements that the State or Tribe must meet while it is in provisionally consistent status.

1.2.7 Listing of current State statuses

- The current statuses assigned to States and Tribes are available as follows:
 - [Appendix 2: Statuses Applied to States and Tribes](#)
 - On the Internet at http://www.aphis.usda.gov/animal_health/animal_dis_spec/cattle.
 - From local VS Area Offices.
 - From the National Tuberculosis Program Coordinator, CHG, VS, APHIS, 4700 River Road Unit 43, Riverdale, MD 20737.

1.3 Reporting

Reporting requirements allow States and Tribes to document continual adherence to their animal health plans and describe occurrences of disease in a timely manner. Compliance with these reporting requirements is a primary determinant of a State's or Tribe's status for the brucellosis and bovine tuberculosis programs. Additionally, the level of transparency and

accountability provided by these reports will contribute to the success and acceptance of these programs. Report templates are available in [Appendix 3: Annual Report Template](#)

1.3.1 Submission of annual report

While APHIS prefers States and Tribes to submit annual reports electronically to the AD, they can submit a paper copy to the [AD](#).

1.4 Program compliance reviews

This Program Standards document provides guidelines specific to reviews of the brucellosis and bovine tuberculosis programs within a State or Tribe. In addition, the guidelines for conducting reviews as described in VS Memorandum 515.1 will apply.

1.4.1 Convening a program compliance review

- APHIS may conduct a review when it is:
 - Evaluating compliance with an animal health plan.
 - Considering redesignating status.
 - Considering establishing, changing, or terminating a recognized management area.
- A State or Tribe may request that APHIS conduct a review to address specific objectives. However, the decision to conduct a review, the scope of the review, and the membership of a review team are at APHIS' discretion.
- Reviews may be conducted on an ad-hoc basis for any of the reasons specified above. Periodic reviews may be conducted in States and Tribal lands with recognized management areas.
- APHIS will define the scope and identify specific objectives for each review. A single review may address the brucellosis program, the bovine tuberculosis program, or both programs. Typically, the objectives will focus on evaluating specific categories within the animal health plan, such as surveillance, risk mitigation measures, and management and response activities.
- APHIS may conduct a review as either a site visit or a documentation review. A site visit is conducted on location in the State or Tribe under review. A documentation review uses a variety of communication methods including email correspondence, conference calls, and webinars, but does not require travel for the review team members. Both types of reviews include interviews of local animal health and wildlife

authorities and review of documents such as correspondence, program records, and reports. However, site visits also allow for direct observations of activities described in the animal health plan. APHIS' selected approach for a program review will depend on the complexity of the review's scope and objectives as well as the availability of funding for travel.

1.4.2 Review team membership

- A review team consists of two or more members. APHIS will determine the size and composition of a review team based on the scope and objectives of the program review.
- APHIS will designate an employee to serve as the team leader for the program review.
- APHIS will select members of a review team based on their unique technical and scientific competencies to meet the objectives of the program review.
- Review team members may be representatives of Federal, State, and Tribal governments. Industry personnel may also be solicited for specific, technical advice as part of a review. The types of representatives and individual members may vary across review teams.

1.4.3 Review reports and recommendations

- The review team leader will prepare a written report summarizing the major findings and recommendations of the review.
- APHIS may make review reports publicly available.

Element 2: Recognized Brucellosis or Bovine Tuberculosis Management Areas

Regulatory authority: 9 CFR 76.5 Recognized management areas

Background

A brucellosis or bovine tuberculosis management area is defined within the regulations as:

A clearly delineated geographical area in which a State or Tribe has detected brucellosis or bovine tuberculosis, has determined that there is a risk of transmission of brucellosis or bovine tuberculosis to program animals, and has taken or proposes to take measures to control the spread of the brucellosis or bovine tuberculosis within and from the area and/or to eradicate the disease within the area.

The regulations also contain the conditions under which APHIS would recognize the establishment or termination of a management area. They provide a structure that ensures consistency in establishment and termination of recognized management areas among States and Tribes. The regulations also require States and Tribes to document and report on the surveillance and mitigation actions performed within the management area to detect, mitigate, and prevent disease spread within or from the management area. This ensures transparency regarding the application and maintenance of these measures.

If management areas for brucellosis and bovine tuberculosis overlap within a State or Tribe, and the epidemiological assessment, surveillance and monitoring activities, mitigation measures, and animal identification requirements for the two management areas conflict, the more stringent of the activities, measures, and requirements apply to that overlapping area. In addition, any disease-specific measures that are unique to either of the two management areas must continue to be maintained in the overlapping area.

2.1 Requirements for recognition of a management area

2.1.1 Submission requirements

In support of a request to APHIS to recognize a management area that the State or Tribe has established, the State or Tribe must submit the information outlined below.

Category 1. Geographical description

Submission requirements

- A. A description of the geographical area that the State or Tribe requests to be recognized as a management area.
- B. The description of the boundaries of the management area should use commonly recognized topographical features (e.g., rivers, mountain ranges, roads, etc.). Coordinates or geopolitical boundaries should be provided instead of topographical features only if a topographical description is not possible. The description may include both topographical features and coordinates or geopolitical boundaries.

Evaluation criteria

- A. The boundaries of the management area are continuous and uninterrupted.
- B. Any topographical features listed in the description are clear and unambiguous.

Category 2. Determination of Boundaries

Submission requirements

- A. A description of the assessments and activities that the State or Tribe has conducted to determine the boundaries of the management area. This must include, at a minimum, descriptions of, dates for, and data regarding the following:
 - 1. Epidemiological investigations (including human and wildlife components, if any).
 - 2. Surveillance activities within the management area to determine or further delineate the sources of brucellosis and/or bovine tuberculosis.
 - 3. Surveillance activities outside the boundaries of the management area.
- B. A description of the means by which the State or Tribe will continue to ensure that the boundaries of the management area remain up-to-date. This must include a description of and timelines for each of the following:
 - 1. Epidemiological investigations (including human and wildlife components, if any).
 - 2. Surveillance activities within the management area to determine or further delineate the sources of brucellosis and/or bovine tuberculosis.

3. Surveillance activities outside of the management area at a rate sufficient to detect brucellosis or bovine tuberculosis infection in program animals that originate from or are otherwise related to the management area.

Evaluation criteria

- A. The assessments and activities provide assurances that the boundaries of the management area continually reflect current epidemiological knowledge about the extent of disease and risk of transmission of disease within and from the area.

Category 3. Sources of disease and assessment of disease spread

Submission requirements

- A. A description of the known sources of brucellosis or bovine tuberculosis that pose a risk of disease introduction into program animals within and surrounding the management area, and an assessment of the likelihood of spread of brucellosis or bovine tuberculosis from these sources to program animals. This must include:
1. The approximate number of herds, individual program animals, and susceptible wildlife populations within the management area¹ and in the area surrounding the management area, as this surrounding area is determined in consultation with the epidemiologist designated by the District Director.
 2. The number of affected herds or wildlife populations detected within the management area since the first investigation or surveillance activity specified by the State or Tribe in their request was conducted, and the approximate number of animals in these herds or source populations.
 3. The approximate prevalence of brucellosis or bovine tuberculosis within these herds and wildlife populations during that time.
 4. The potential for exposure of program animals to these known herds or wildlife populations.
 5. Any factors, other than mitigation measures maintained by the State or Tribe, which may influence this potential for exposure (e.g., movements or movement patterns of affected or potentially affected animals or susceptible animals, seasonal variations, etc.).
 6. An assessment (qualitative or quantitative) of the likelihood of transmission from known affected herds or wildlife populations to program animals within and surrounding the management area.

Evaluation criteria

- A. All the required information has been submitted.

¹ If a producer has herds both within and outside of the management area, both should be counted towards this total, unless there is no commingling of these herds.

Category 4. Risk Mitigation Measures

Submission requirements:

A description of the measures that the State or Tribe has implemented or would implement including a timeline for implementation to mitigate the risk that program animals within the State or Tribal lands will become infected with brucellosis or bovine tuberculosis, and the means by which the State or Tribe has monitored and enforced or plans to monitor and enforce these measures. Measures must include:

1. Conditions for the movement of program animals from the management area.
2. Herd testing of at least a targeted representative sample of herds of program animals within the area.
3. Change-of-ownership testing for all test-eligible program animals residing within the management area.
4. For management areas for brucellosis, the measures must include an official brucellosis vaccination program (see Official brucellosis vaccination program).

B. Suggested measures also include:

1. Fencing to limit wildlife and livestock exposures.
2. Vaccination of wildlife.
3. Wildlife habitat improvement.
4. First-point testing.
5. Individual herd plans.
6. Biosecurity measures.
7. Quarantined feedlots and quarantine pens.

B. The dates when mitigations were implemented.

C. A timeline for implementing future mitigations.

Evaluation criteria

- A. All the required information has been submitted.

Category 5. Laws and regulations

Submission requirements

- A. A citation of or hyperlink to the statute or regulation explicitly authorizing the State or Tribe to establish management areas. (This statute or regulation may refer to zones, monitoring areas, or another term, if the statute or regulation defines such areas at least as broadly as the definition of management area within APHIS' regulations.)
- B. In the absence of a statute or regulation explicitly authorizing the State or Tribe to establish management areas, a citation of or hyperlink to the statute or regulation under which the State or Tribe claims authority to establish a management area.
- C. A citation of or hyperlink to the statute or regulations authorizing the mitigation measures the State or Tribe has taken or plans to take within the management area.

Evaluation criteria

- A. All the required information has been submitted.
- B. The statutes or regulations cited by the State or Tribe provide a basis for establishing a management area.
- C. The statutes or regulations provide clear authority for the activities and measures the State or Tribe has taken or plans to take within the management area.

Category 6. Personnel

Submission requirements

- A. A description of the personnel that the State or Tribe has used or plans to use in order to implement or perform activities or maintain measures associated with the management area. The description must include:
 - 1. The name, contact information, and affiliation (e.g., State Department of Agriculture, State Department of Natural Resources, Tribal Natural Resources

Division) of the person within the State or Tribe who will assume responsibility for implementation and performance of activities and maintenance and enforcement of measures associated with the management area.

2. The name, contact information, and affiliation (e.g., Federal or State Department of Agriculture, State Department of Natural Resources, third party contractor working under a cooperative agreement, personnel from neighboring States or Tribes operating under an agreement with the State, Tribe, etc.) of all personnel assigned to implementation and performance of activities and maintenance and enforcement of measures associated with the proposed management area.

3. The role or roles assigned to these personnel.

Evaluation criteria

- A. All the required information has been submitted.
- B. The information demonstrates that the State or Tribe has sufficient personnel to implement and perform activities and maintain and enforce measures associated with the management area.

Category 7. Official Identification

Submission requirements

- A. Information demonstrating that all program animals that are moved from the management area are or will be required to be officially identified prior to movement. This must include either:
 - 1. A citation to a statute or regulation requiring all cattle, bison, and captive cervids that are moved from the management area to be identified with an official eartag before movement; or
 - 2. In the absence of such a statute or regulation, evidence showing that such a requirement will be implemented (including a timeline for implementation) and enforced.

Evaluation criteria

- A. All the required information has been submitted.

2.1.2 Official brucellosis vaccination program

2.1.2.1 Procedures for whole-herd vaccination

Whole-herd vaccination entails the vaccination of all female cattle and/or bison 4 months of age or over, including animals older than the age for official calfhood vaccination, when the entire herd is authorized by the State or Tribal animal health official and by the APHIS AD to be vaccinated with an approved dose of an official brucellosis vaccine. All animals included in the whole-herd vaccination must be officially identified in accordance with the standards for official brucellosis vaccinates.

- Whole-herd vaccination should be reserved for use in cattle and/or bison herds that are at high risk of becoming infected with brucellosis and should be initiated only with permission from the State or Tribal and Federal officials directly responsible for Program activities in the State or Tribe where the herd is located.
- An individual herd plan that specifies the whole-herd vaccination procedures to be used must be developed and agreed to. The plan should also include other recognized procedures for preventing the introduction of brucellosis into the herd and for controlling its spread if it does occur.
- As part of the individual herd plan for whole-herd vaccination:
 - All eligible calves must be calfhood-vaccinated and officially identified in accordance with the standards for identification of official brucellosis vaccinates.
 - All female cattle and/or bison vaccinated as adults must be officially identified in accordance with the standards for identification of official brucellosis vaccinates.

2.1.2.2 Procedures for calfhood brucellosis vaccination

To be an official calfhood vaccinee, the vaccinated bovine or bison calf must be a bovine or bison female that, as a calf, was vaccinated with an official brucellosis vaccine, between the appropriate age limits, by a State, Tribal, or Federal representative or an accredited veterinarian using the approved calfhood brucellosis vaccination procedure. The animal must have been properly identified as an official calfhood vaccinee by official eartag and vaccination tattoo and must have been reported on the appropriate certificate to the appropriate State, Tribal, or Federal animal health agency for that State or Tribe.

- Official calfhood vaccinates must be **all** of the following:
 - Female
 - Vaccinated with an official brucellosis vaccine
 - Vaccinated by a State, Tribal, or Federal animal health representative or an accredited veterinarian
 - Vaccinated between 4 months and 1 year of age (120 through 365 days)
 - Given a subcutaneous 2-ml dose of *Brucella abortus* Strain RB51 vaccine containing at least 10 billion and not more than 34 billion live organisms
 - Permanently identified as a calfhood vaccinate in accordance with the standards for identification of official brucellosis vaccinates
 - The accredited veterinarian, State or Tribal representative, or APHIS representative who performs the vaccination must forward, at the time of vaccination, a completed official vaccination certificate (VS Form 4-24 or VS Form 4-26) which identifies each animal vaccinated to the State or Tribal animal health official of the State or Tribe in which the animal was vaccinated.

- Identifying official calfhood vaccinates: Calfhood-vaccinated animals must be permanently identified as official calfhood vaccinates by official eartag and by tattoo in accordance with the following guidelines:
 - Official eartag:
 - If the animal is already identified with an official eartag before vaccination, an additional official eartag is not required.
 - Official eartags must be applied to the right ear or the location specified for a particular type of official eartag (example: 840 eartags are placed in the left ear).
 - Individual animal registration tattoos or individual animal registration brands may be used for identifying animals in place of official brucellosis vaccination eartags if the cattle and/or bison are registered by breed associations recognized by VS.
 - Tattoo: The tattoo is also required, in addition to the official eartag.
 - For *Brucella abortus* Strain RB51 vaccinates, the tattoo must include the U.S. Registered Shield and “V,” which will be preceded by a letter “R” and followed by a number corresponding to the last digit of the year in which the vaccination was done.
 - Vaccination tattoos must be applied to the right ear.
 - Official calfhood vaccinates are allowed to be re-tattooed by an accredited veterinarian designated by the State or Tribal Veterinarian, or by a Federal, State, or Tribal representative, provided that:
 - The identification of the vaccinated animal(s) is verified by official records maintained in State, Tribal, or Federal offices.
 - Prior approval for re-tattooing is obtained from the State or Tribal Veterinarian.
 - The re-tattooing produces the original tattoo given at the time of official calfhood brucellosis vaccination.

- The accredited veterinarian, State or Tribal representative, or APHIS representative who performs the re-tattooing must forward, at the time of re-tattooing, a completed official vaccination certificate (VS Form 4-24 or VS Form 4-26) which identifies each animal re-tattooed to the State or Tribal animal health official of the State or Tribe in which the animal was vaccinated.

2.1.2.3 Procedures for adult vaccination

To be an official adult vaccinate, the vaccinated animal must be a bovine or bison female that, as part of a herd approved for whole-herd vaccination or an individual animal approved for adult vaccination as authorized jointly by the State or Tribal animal health official and the Veterinarian in Charge, was vaccinated with an official *Brucella* vaccine at an age older than that permitted for calfhood vaccination. Adult vaccinated animals must be inoculated by a State, Tribal, or Federal representative or an accredited veterinarian using the approved procedure. At vaccination, the animal must have been properly identified as an official adult brucellosis vaccinate and must have been reported on the appropriate form to the State, Tribal or Federal animal health agency in that State or Tribe.

- Official adult vaccinates must be **all** of the following:
 - Part of a herd approved for whole-herd vaccination or an animal approved for individual vaccination at the time of vaccination
 - Female cattle and/or bison vaccinated at an older age than the maximum age approved for calfhood vaccination
 - Vaccinated with an official brucellosis vaccine
 - Vaccinated subcutaneously with an approved 2-mL dose of *Brucella abortus* Strain RB51 vaccine containing at least 1 billion live organisms
 - Vaccinated by a State, Tribal, or Federal animal health representative or by an accredited veterinarian as instructed by the State or Tribal animal health official and the APHIS AD
 - Identified as an official adult vaccinate as described in the subsection below
 - The accredited veterinarian, State or Tribal representative, or APHIS representative who performs the vaccination must forward, at the time of vaccination, a completed official vaccination certificate (VS Form 4-24 or VS Form 4-26) which identifies each animal vaccinated to the State or Tribal animal health official of the State or Tribe in which the animal was vaccinated.
- Identifying official adult vaccinates: Animals that have been vaccinated over calfhood age as part of authorized whole-herd vaccination plans or as an individual animal approved for adult vaccination must be permanently identified as vaccinates as follows:
 - Official eartag:
 - If the animal is already identified with an official eartag before vaccination, an additional official eartag is not required.

- Official eartags must be applied to the right ear or the location specified for a particular type of official eartag (example: 840 eartags are placed in the left ear).
- If the cattle or bison are registered by a breed association recognized by VS, individual animal registration tattoos or individual animal registration brands may be used for identifying animals in place of official eartags.
- Tattoo: The tattoo is also required, in addition to the official eartag.
 - For *Brucella abortus* Strain RB51-vaccinated animals, the “AV” in the tattoo is to be preceded by the letter “R” and followed by a number corresponding to the last digit of the year in which the vaccination was done.
 - Adult-vaccinated cattle and/or bison must be identified by an official “AV” (adult vaccination) tattoo in the right ear.

2.2 Submission process

2.2.1 Submission process for States and Tribes without an existing management area

If the State or Tribe does not already have an APHIS-approved animal health plan, the State or Tribe must submit a request for APHIS recognition of the management area as part of its initial animal health plan. If the State or Tribe already has an APHIS-approved animal health plan, the request must be made through an amended animal health plan (see Appendix 8: Amendment to Animal Health Plan for Recognition of a Management Area). The categories of information specified for the management area should be appended to the animal health plan (see [Animal health plan](#)). States and Tribes that submit a request for recognition of a management area should also review all categories of information within the animal health plan to ensure that no other amendments to the plan are necessary to accommodate the request for recognition of the management area.

2.2.3 Submission process for States with areas covered by an existing brucellosis management plan or with zones for bovine tuberculosis

Certain States have areas covered by brucellosis management plans or have zones established for bovine tuberculosis. If a State or Tribe wants such an area or zone to continue to be recognized as a management area under this new program, the State or Tribe must submit a proposal for recognition of the area. However, this request only needs to contain those categories of information that the State or Tribe has not already submitted to APHIS. For example, as part of its request to have a zone for tuberculosis recognized as a management area, a State or Tribe would not have to resubmit information showing that it has legal authority to establish the zone, or information regarding clinical and epidemiological surveillance activities conducted within the zone.

2.3 APHIS review and approval

APHIS will review a request for recognition of a management area according to the process for review or amendment of an animal health plan. This notice-based approach is also described in 9 CFR 76.2 of the regulations (see [Animal health plan](#)).

If a State or Tribe submitted a request for recognition of a management area to APHIS as part of an initial animal health plan (i.e., not an amendment to an APHIS-approved plan) the request will be reviewed as part of the initial animal health plan. If a State or Tribe submits a request for recognition of a management area as an amendment to an approved animal health plan, APHIS will review the plan for completeness. If it is complete, APHIS will initiate review and evaluation of the request. This may include sharing a copy of the request with persons for technical review and comment. If, based on its review, APHIS determines not to approve the request for recognition of a management area, APHIS will contact the State or Tribe and set forth the deficiencies identified in the request that preclude APHIS from approving the request. APHIS will not recognize a management area in a State or on Tribal lands if it determines not to approve that State or Tribe's animal health plan.

2.3.1 Proposal to approve unconditionally

Based on its review, APHIS may propose to approve a State or Tribal request unconditionally, or on the condition that the State or Tribe implement certain provisions of its request within a specified period of time that it cannot implement immediately upon approval of the request. In either instance, APHIS will publish a notice in the *Federal Register* announcing receipt and proposed approval of the request and making the request available for public review and comment. State or Tribes submitting a request should be aware that the request will be publicly available.

Following a notice proposing unconditional approval of a request for recognition of a management area, if no comments are received on the notice, or if the comments received do not affect APHIS' conclusion that the request may be approved unconditionally, APHIS will publish a subsequent notice in the *Federal Register* announcing that the request has been approved unconditionally, and granting APHIS recognition of the management area.

2.3.2 Proposal to approve conditionally

If the comments received on the notice suggest that the request should be approved, but that State or Tribe cannot implement certain provisions of its request immediately upon APHIS recognition of the management area, and, after reviewing the information, APHIS agrees, APHIS will publish a subsequent notice in the *Federal Register* announcing that the request has been approved conditionally. The notice will also specify the provisions of the request that

APHIS has determined cannot be implemented immediately and the time period in which they must be implemented.

If the comments received suggest that APHIS should not recognize the management area, and, after reviewing the information, APHIS agrees, APHIS will publish a subsequent notice in the *Federal Register* presenting the information supplied by the commenters to the public, and setting forth the deficiencies identified in the request that preclude APHIS from approving the request.

Following a notice proposing conditional approval of a request for APHIS recognition of a management area, if no comments are received on the notice, or if the comments received do not affect APHIS' conclusion that the request may be approved on the condition that the State or Tribe implement certain provisions of its request within a specified period of time that it cannot implement immediately upon approval of the request, APHIS will publish a subsequent notice in the *Federal Register* announcing that APHIS is recognizing the management area conditionally. The notice will also specify the provisions of the request that APHIS has determined cannot be implemented immediately and the time period in which they must be implemented.

If the comments received suggest that the request should not be approved, and, after reviewing the information, APHIS agrees, APHIS will publish a subsequent notice in the *Federal Register* presenting the information supplied by the commenters to the public, and setting forth the deficiencies identified in the request that preclude APHIS from conditionally recognizing the management area.

If APHIS approves a State or Tribal request for recognition of a management area on the condition that it implement certain provisions of its request within a specified period of time that it cannot implement immediately upon approval of the request, APHIS will publish a subsequent notice in the *Federal Register* announcing whether the State or Tribe has implemented these provisions within that period of time.

If the State or Tribe has implemented the provisions, the notice will also announce that APHIS now considers the request for recognition of a management area unconditionally approved. If the State or Tribe has not implemented the provisions, the notice will also announce that APHIS has withdrawn conditional recognition of the management area.

2.3.3 Amendments

2.3.3.1 Amendments initiated by APHIS

If APHIS determines that the measures in the request for recognition of the management area no longer addresses the risk of spread of brucellosis or bovine tuberculosis, APHIS will make ongoing recognition of the management area contingent on the State or Tribe amending the

provisions of the recognized management area in the manner that APHIS approves of. The amended provisions must be submitted to APHIS physically or electronically via the [AD](#).

2.3.3.2 Amendments initiated by a State or Tribe

If a State or Tribe wishes to expand or contract the geographical boundaries of a recognized management area, or determines that any information in its request for a management area has substantively changed, the State or Tribe must submit amendments to its animal health plan that reflect these changes electronically or physically to the AD. Amendments will be subject to the review process specified in this section.

2.4 Annual reporting

In addition to the annual reporting requirements contained in 9 CFR 76.4, States and Tribes with recognized management areas must submit a separate annual report for each recognized management area in the State or Tribe with their annual animal health plan report.

2.5 Termination of a management area

A State or Tribe may elect to terminate a recognized management area for several reasons, including:

- Eradication of brucellosis or bovine tuberculosis within the management area.
- A determination that brucellosis or bovine tuberculosis has been transmitted from animals within the management area to animals outside the management area.

To request APHIS recognition of termination of the management area, a State or Tribe must submit an amended animal health plan to APHIS in accordance with 9 CFR 76.2 (see [Animal health plan](#)), striking through the appendix for the recognized management area.

If the termination is based on eradication, the State or Tribe should provide the information on which it relied to determine that brucellosis or bovine tuberculosis has been eradicated within the management area. If the termination is based on the spread of brucellosis or bovine tuberculosis to animals outside the management area, the State or Tribe must also amend the animal health plan to specify additional mitigation measures for program animals moved interstate.

APHIS will review all requests for termination of a recognized management area according to the procedures in 9 CFR 76.2 (see [Animal health plan](#)) for review of an amendment to an animal health plan.

After review, APHIS will communicate its determination regarding the termination in the *Federal Register*. If the termination is based on a lack of resources to maintain the management area or the spread of brucellosis or bovine tuberculosis to animals outside the management area, and APHIS determines that the additional mitigation measures specified by the State or Tribe are not adequate, this may also involve redesignation of the State or Tribe to provisionally consistent or inconsistent status.

APHIS may also terminate recognition of all management areas within a State, without a request from a State or Tribe to do so, if the Agency determines that the State or Tribe has failed to implement or maintain measures specified in its request for recognition of any management area within the State or Tribe. In such cases, APHIS will contact the State or Tribe regarding this termination and the reasons for it. Subsequently, APHIS will publish a notice in the *Federal Register* announcing termination of the recognized management area(s), and re-designating the State or Tribe as an inconsistent State or Tribe.

Further, when APHIS downgrades a State or Tribe to inconsistent status, we will also terminate recognition of all management areas within that State or Tribe.

2.6 APHIS program reviews of recognized management areas

APHIS may conduct an ad-hoc review of a State or Tribe's implementation, maintenance or enforcement of management area activities at any point. Reviews will typically be conducted when the State or Tribe requests recognition of the management area, when the State or Tribe wishes to change the boundaries of the area or substantively modify activities conducted within the area, or when the State or Tribe requests recognition of its termination of the recognized management area (See [Program compliance reviews](#))

Element 3: Brucellosis and Bovine Tuberculosis Surveillance

Regulatory authority: 9 CFR 76.6 Surveillance requirements.

Background

Surveillance is a key activity in the eradication of diseases. The objectives for any surveillance activity are:

- Rapidly detecting brucellosis and bovine tuberculosis in program species.
- Estimating the magnitude of these infections (prevalence and incidence).
- Measuring progress toward regulatory goals (eradication and control).
- Providing metrics to aid in evaluating compliance with program standards.
- Giving stakeholders and decision makers timely and relevant actionable information.

The national surveillance approach is outlined in the national surveillance plans for brucellosis and bovine tuberculosis programs and describes the minimal surveillance that will occur. On occasion, APHIS may request that States or Tribes supplement this national surveillance with their own routine surveillance. However, most State and Tribal surveillance activities will be those that are required when the State or Tribe determines enhanced surveillance is needed to target a potential disease risk. These targeted surveillance activities will be outlined in the animal health plans.

3.1 National surveillance

APHIS and the States and Tribes will collaborate to conduct slaughter and other routine surveillance as defined in the National Surveillance Plans for program animals.

3.1.1 Surveillance for brucellosis in cattle and bison

- Surveillance will be conducted in accordance with the National Brucellosis Surveillance Plan located at http://www.aphis.usda.gov/animal_health/animal_diseases/brucellosis/.
- The National Brucellosis Surveillance Plan is designed to detect brucellosis at a minimum level of 1 infected animal per 100,000 animals (0.001 percent prevalence) in the national dairy and beef cattle and domestic bison populations, combined, with a 95 percent confidence.

- All Tribes that wish to be regulated as distinct entities and States must participate in the National Brucellosis Surveillance Plan. Alternatively, a State or Tribe may develop an alternative strategy to the National Brucellosis Surveillance Plan (see “Evaluation criteria and reporting requirements for States and Tribes implementing an alternative surveillance plan”).

3.1.2 Surveillance for brucellosis in captive cervids

- Surveillance information for brucellosis in captive cervids will be captured by testing all captive cervids destined for interstate movement for bovine brucellosis before such movement occurs, unless the cervids originate from a currently accredited herd or are destined for immediate slaughter.
- Currently, there are no routine slaughter surveillance standards for brucellosis in captive cervids. However, VS encourages collection and submission of blood samples for brucellosis testing from captive cervids at or destined for slaughter.

3.1.3 Surveillance for bovine tuberculosis in cattle and bison

- Surveillance will be conducted in accordance with the National Tuberculosis Surveillance Plan located at http://www.aphis.usda.gov/animal_health/animal_diseases/tuberculosis/.
- The National Tuberculosis Surveillance Plan is designed to detect bovine tuberculosis at a level of 3 infected animals per 1 million animals (0.0003 percent prevalence), among the U.S. adult cattle and bison population with a 95 percent confidence.
- All Tribes that wish to be regulated as distinct entities and States must agree to participate in the National Tuberculosis Surveillance Plan. Alternatively, States or Tribes may develop an alternative strategy to the National Tuberculosis Surveillance Plan (see “Evaluation criteria and reporting requirements for States and Tribes implementing an alternative surveillance plan”).
- In addition to participating in the National Tuberculosis Surveillance Plan, each State or Tribe must implement a system to monitor the caudal fold test response rate of each BTB QAV and regulatory veterinarian authorized to conduct official tuberculin tests. A response rate of less than one responder for each range of caudal fold tuberculin (CFT) tests conducted as specified in [Appendix 9: Performance Standards for Caudal Fold Tuberculin Testing](#), after 300 animals have been tested, must be addressed and appropriate action taken and documented.

3.1.4 Surveillance for bovine tuberculosis in captive cervids

- Surveillance information for bovine tuberculosis in captive cervids will be captured by testing all captive cervids destined for interstate movement for bovine tuberculosis before such movement occurs, unless the cervids originate from a currently accredited herd or are destined for immediate slaughter.
- Currently, there are no routine slaughter surveillance standards for bovine tuberculosis in captive cervids. However, VS encourages the submission of granulomas when observed.

3.1.5 Evaluation criteria and reporting requirements for States and Tribes implementing an alternative surveillance plan

3.1.5.1 Approval

For States and Tribes that are proposing an alternative brucellosis or bovine tuberculosis surveillance plan, the States and Tribes must demonstrate that the proposed alternative plan meets or exceeds the level of disease detection estimated for the National Surveillance Plans.

- VS approval will require concurrence of the AD, District Director, National Surveillance Unit (NSU) director, and CHG director (or their designees).
- Once approved, the plan shall be incorporated into the State's or Tribe's animal health plan.

3.1.5.2 Reporting

States or Tribes must:

- Submit annual reports of the assessments or activities conducted by the State or Tribe as part of their alternative surveillance plan. These reports must be sufficiently specific to allow APHIS to determine whether the alternative plan continually meets or exceeds the level of disease detection estimated for the National Surveillance Plans.
- Submit quarterly and annual reports containing all information requested by the Administrator.

3.2 Targeted surveillance

3.2.1 Requirements for animal health plans

- The animal health plan must adequately address source and at-risk populations through testing, movement controls, slaughter surveillance, or other appropriate risk mitigating management practices.
- The animal health plan will define at-risk populations and source populations (both domestic and wildlife) and the manner of surveillance to be used in these populations.

3.2.2 Recommendations for animal health plans

APHIS recommends that States and Tribes consider the use of the following surveillance streams when developing targeted surveillance:

- Change-of-ownership testing
- Testing at livestock markets (specifically for brucellosis)
- Testing for shows and exhibitions
- Area testing
- Targeted slaughter surveillance that is designed to exceed the sample size described in the National Surveillance Plan for specific slaughter establishments

3.2.3 Surveillance of source populations

3.2.3.1 Livestock

- Surveillance and testing requirements for source populations in livestock (affected herds) are specified within the “[Epidemiological Investigations and Affected Herd Management](#)” and “Interstate Movement Requirements” sections of the program standards and must be addressed in affected herd management plans.

3.2.3.2 Wildlife

It is recommended, but not required, that a State or Tribe conduct surveillance in wildlife in areas where brucellosis or bovine tuberculosis has been detected in a dairy, beef, bison, or

captive cervid herd and is not previously known to be present in wildlife (see “[Epidemiological Investigations and Affected Herd Management](#), Wildlife).

- If a State or Tribe has identified a known source population for brucellosis or bovine tuberculosis within wildlife in its animal health plan and determined that this source population presents a risk of transmitting brucellosis or bovine tuberculosis to program animals, the State or Tribe must conduct ongoing surveillance activities within that source population sufficient to detect brucellosis or bovine tuberculosis in an animal within that source population. States and Tribes should:
 - Base the targeted level of detection for brucellosis or bovine tuberculosis on:
 - The best-known science and epidemiology of the disease in the affected wildlife population;
 - Current relevant population data; and
 - Best available diagnostic technology.
 - Conduct brucellosis or bovine tuberculosis surveillance in such a manner as to provide routine surveillance within the wildlife species of concern under State or Tribal control.
 - Use Guidelines for Surveillance of Bovine Tuberculosis in Wildlife, located at http://www.aphis.usda.gov/animal_health/animal_diseases/tuberculosis/, as a reference document. States and Tribes can request sample size calculators and consult with NSU concerning wildlife surveillance.
- States and Tribes should provide their goal for a detection rate and describe their sample size calculations for their planned surveillance activities. A State or Tribe may request assistance from VS to develop these activities.

3.2.4 Surveillance of at-risk populations

- If a State or Tribe has identified a source population for brucellosis or bovine tuberculosis in the State or Tribe in its animal health plan and has determined that this source population presents a risk of transmitting brucellosis or bovine tuberculosis to program animals, the State or Tribe must conduct ongoing surveillance within the at-risk program animals.
- The minimum surveillance activities for brucellosis and bovine tuberculosis include annual herd testing of all, or an APHIS-determined statistically significant sample, of herds.
- States and Tribes should provide their goal for a detection rate and describe their sample size calculations for their planned surveillance activities. A State or Tribe may request assistance from VS to develop these activities.

3.2.5 Surveillance within recognized management areas

- States and Tribes must conduct surveillance within a recognized management area in the manner specified within the section of their animal health plan that pertains to the recognized management area.
- The surveillance strategy must be able to detect brucellosis or bovine tuberculosis within program animals in the management area, as well as in the area surrounding the management area. The scope of this surrounding “buffer” area must be determined in consultation with a designated disease epidemiologist.
- The minimum surveillance activities for brucellosis and bovine tuberculosis within the management area must include:
 - An annual herd test of all, or an APHIS-determined statistically significant sample, of herds.
 - Change of ownership testing or market testing of test-eligible program animals.
- Surveillance activities for brucellosis or bovine tuberculosis conducted in the area surrounding the management area must be sufficient to detect brucellosis or bovine tuberculosis infection in animals that originate from or are otherwise related to the management area.
- States and Tribes should provide their goal for a detection rate and describe their sample size calculations for these planned surveillance activities. A State or Tribe may request assistance from VS to develop these activities.

3.2.6 Surveillance as part of a disease response effort

Surveillance and testing requirements for disease response efforts are specified within the “Epidemiological Investigations and Affected Herd Management” and “Interstate Movement Requirements” sections of this program standards document.

3.2.7 Surveillance standard operating procedures

- The Slaughter Surveillance Procedures Manual is available at http://www.aphis.usda.gov/animal_health/animal_diseases/. This manual describes the details for sample collection and submission, management of approved livestock markets, and forms for all cattle health surveillance programs.

The Food Safety Inspection Service (FSIS) tuberculosis slaughter surveillance awards program is described in VS Guidance Document 6704.1, “Slaughter Surveillance Awards for the Bovine Tuberculosis Program,” and will be updated as needed.

3.3 Surveillance plan submission process

The State or Tribe must submit its plan for targeted surveillance activities or its alternative to the national tuberculosis or brucellosis surveillance plans as part of its animal health plan. If the State or Tribe already has an APHIS-approved animal health plan, the State or Tribe must submit any changes to surveillance activities by amending the animal health plan. Instructions for submitting an amended animal health plan are in 9 CFR 76.2(f) of the regulations (see [Animal health plan](#)).

Element 4: Epidemiological Investigations and Affected Herd Management

4.1 Epidemiological investigation standards

Regulatory authority: 9 CFR 76.7 Epidemiological investigations and affected herd management

Background

When a brucellosis or bovine tuberculosis case-animal is identified, APHIS and the animal health officials from the State or Tribe in which the animal originated will investigate this occurrence. The investigation will include herd-identification and testing to determine disease status of potential source-herds, receiving herds, and adjacent or other contact herds.

4.1.1 Identify and restrict movements within 15 days of identification

The following herds must be identified and their movements restricted within 15 days of identification:

- Any herd, calf raiser, or feedlot which livestock from the brucellosis or bovine tuberculosis-affected herd may have originated in, resided in, or been moved into or through.
- Any herd, calf raiser, or feedlot in which an infected individual animal detected during slaughter or through other means may have originated in, resided in, or been moved into or through.
 - For disease-affected herds, this entails identification of all herds or operations listed above:
 - For 5 years prior to disclosure of infection; or
 - Back to time that infection entered the affected herd as determined by the epidemiologist designated by the District Director in conjunction with CHG specialists.
 - For infected individual animals, this entails identification of all herds or operations back to the herd of birth of the infected animal.
- Any herd, calf raiser, or feedlot having fence line (contiguous) or other contact with the brucellosis- or bovine tuberculosis-affected herd or within a defined area around the brucellosis- or bovine tuberculosis-affected herd as determined by the epidemiologist designated by the District Director in conjunction with CHG specialists.

4.1.2 Determine the test-eligible program animals

Test-eligible program animals include:

- Any herd or calf raiser in which livestock from the brucellosis- or bovine tuberculosis-affected herd or infected individual animal may have originated in or resided in.
- Any herd or calf raiser having fence line (contiguous) or other contact with the brucellosis- or bovine tuberculosis-affected herd or within a defined area around the brucellosis- or bovine tuberculosis-affected herd as determined by the epidemiologist designated by the District Director in conjunction with the CHG specialist.
- Any herd or calf raiser that commingled animals with the affected herd.
- For brucellosis: Test eligible animals are all sexually intact animals 6 months and older, and all program animals that are less than 6 months of age and were not born into the herd, except those program animals that are less than 6 months of age and originated directly from an accredited herd for brucellosis.
- For bovine tuberculosis: Test eligible age for calf raisers is 2 months and older. For other herds described in this section, the age is 12 months and older. Additionally, all animals in a herd that are less than 12 months of age and were not born into the herd are considered test-eligible, except animals that originated directly from an accredited herd for bovine tuberculosis.

All herds having fence line contact with an affected herd that is managed under a test-and-remove protocol must be retested per this section within 6 months after quarantine release of the test-and-remove affected herd.

4.1.3 Epidemiological investigation testing for brucellosis

An accredited or regulatory veterinarian must draw blood for an official brucellosis screening test of all test-eligible animals in the herd.

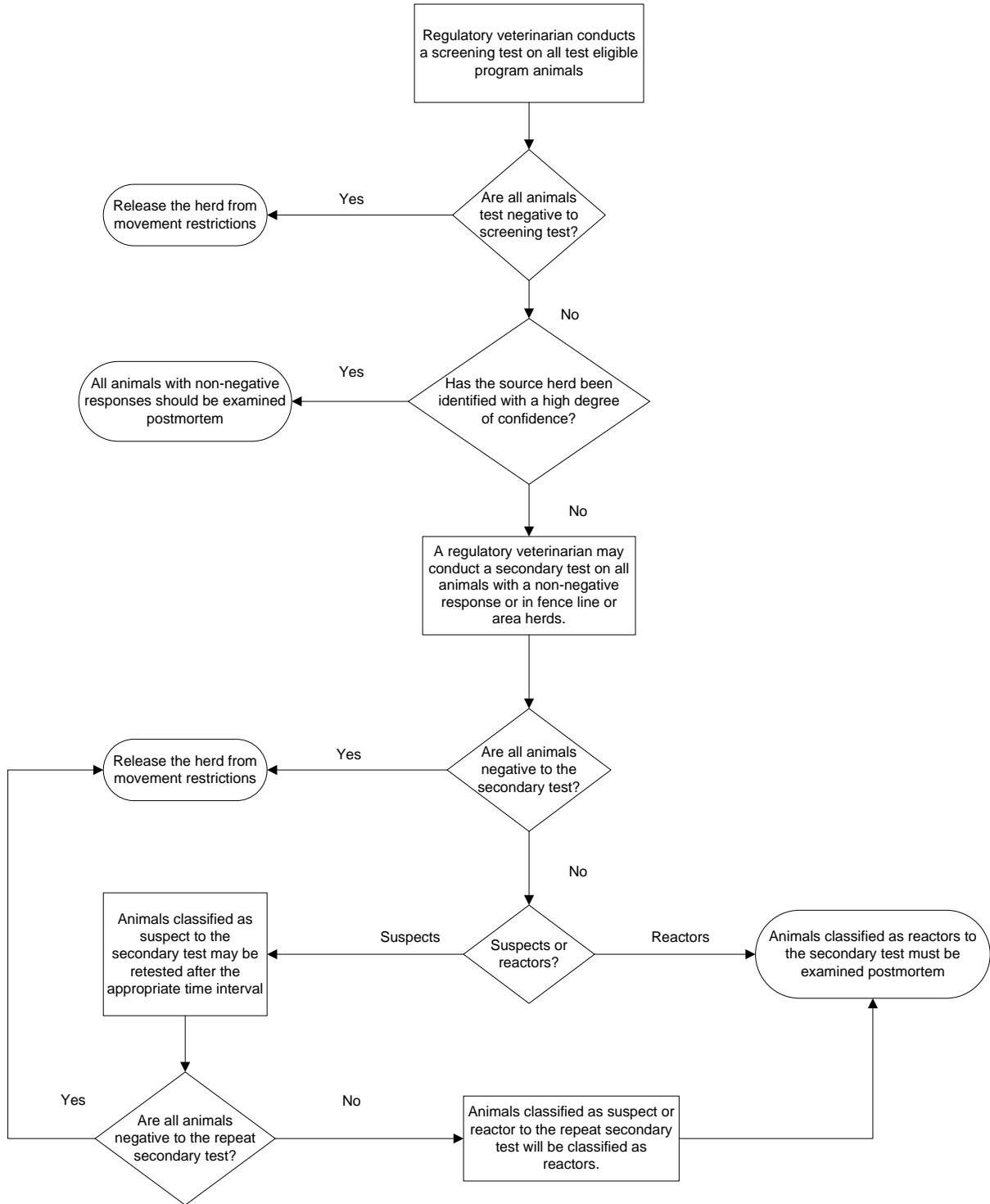
- If all test-eligible animals are negative to the screening test, the herd may be released from movement restrictions.
- A secondary (corroboratory) test may be conducted on all animals with a nonnegative response to the screening test. All animals with a nonnegative response to the secondary test will be classified by the epidemiologist designated by the District Director or CHG specialists and subjected to further testing as deemed necessary by the epidemiologist designated by the District Director to determine their disease status.

4.1.4 Epidemiological investigation testing for bovine tuberculosis

A regulatory veterinarian must conduct an official tuberculosis test of all test-eligible animals in the herd using the screening test. The process is described below and in Figure 2.

- If all test-eligible animals are negative to the screening test, then the herd may be released from movement restrictions.
- If the source herd that provided the infected animal has been identified, then all animals from that herd with responses to the screening test must be examined postmortem.
- If the source herd has not been identified with a high degree of confidence, or the herd is a fence line or area herd, a regulatory veterinarian may conduct a secondary test on all animals with a nonnegative response to the screening test.
- If all animals are negative to the secondary test, then the herd may be released from movement restrictions.
- Animals classified as suspect to the secondary test may be retested after the appropriate time interval. See Laboratory and Diagnostic Test Approval for a description of appropriate intervals.
- Animals classified as suspect to the repeat secondary test will be classified as reactors.
- Animals classified as reactors to the secondary test must be examined postmortem.

Figure 2: Epidemiological investigation testing for bovine tuberculosis



4.1.5 Postmortem examinations

All animals requiring postmortem examination must be slaughtered and their tissues must be submitted to the National Veterinary Services Laboratories (NVSL).

- If there is no evidence of brucellosis or bovine tuberculosis following laboratory examination, then the herd may be released from quarantine. It is recommended to retest the herd in 1 year for brucellosis and 1 year for bovine tuberculosis.
- If NVSL confirms infection, then the herd will be considered as affected and managed accordingly. See [Brucellosis or bovine tuberculosis affected herd management standards](#).

4.1.6 Animals for diagnostic purposes

Any herd or calf raiser in which livestock from the brucellosis- or bovine tuberculosis-affected herd may have been moved into or through must have their disease status determined. If possible, the identified bovine tuberculosis-exposed animals should be necropsied for diagnostic purposes. Indemnity funds *may* be available for this purpose.

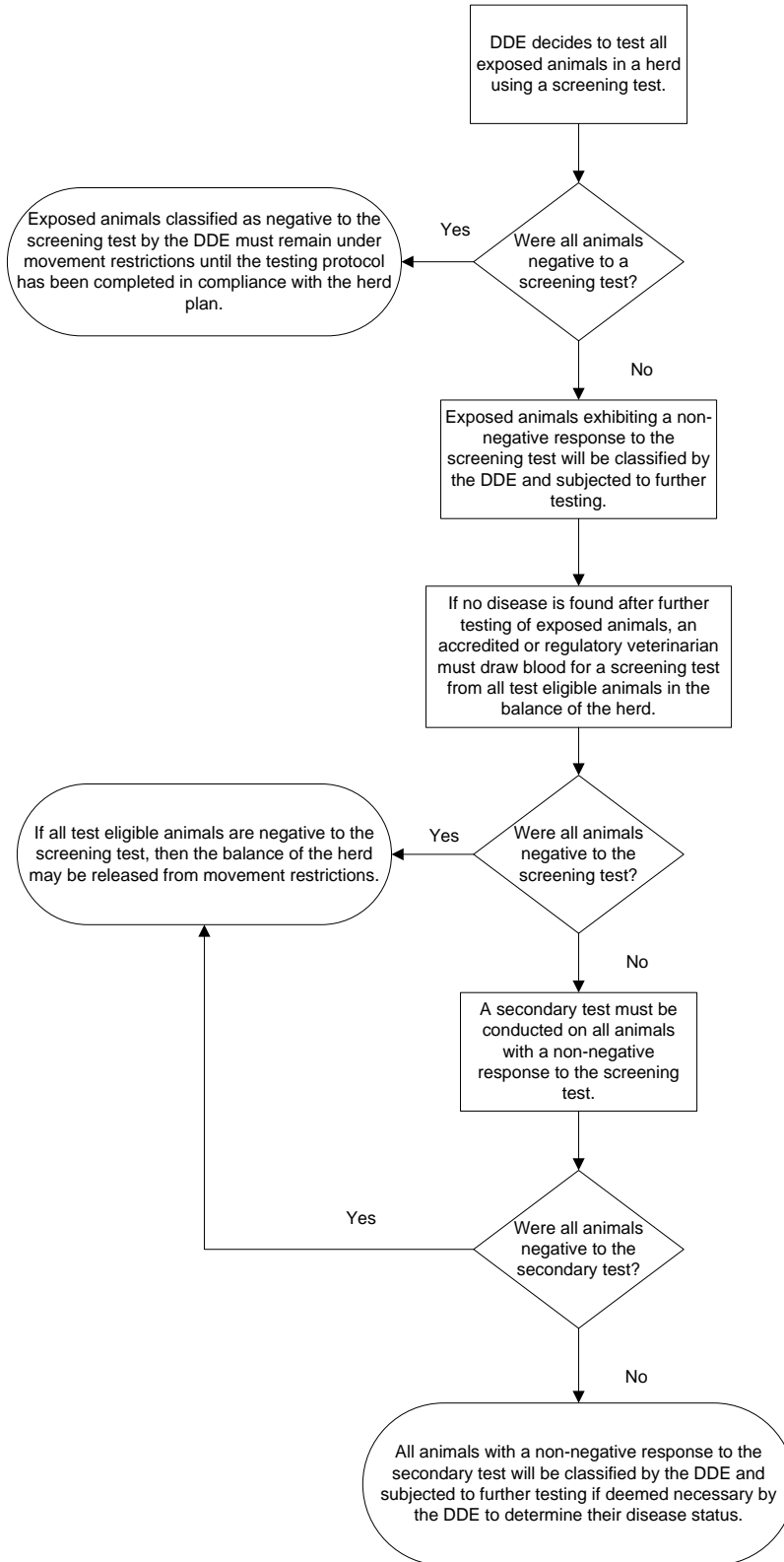
4.1.6.1 Animals for diagnostic purposes: brucellosis

Testing is preferred for diagnosis of brucellosis. Testing of diagnostic exposed animals must be determined by the epidemiologist designated by the District Director. See Figure 3.

- Exposed animals will be tested with a screening test as determined by the epidemiologist designated by the District Director.
- All exposed animals classified as negative by the epidemiologist designated by the District Director must remain under movement restrictions until they have completed a testing protocol in accordance with a herd plan developed for their disease affected herd of origin as indicated in affected herd management standards.
- Any exposed animal exhibiting a non-negative response to the screening test shall be classified by the epidemiologist designated by the District Director and subjected to further testing as deemed necessary.
- If there is no evidence of disease found after further testing of the exposed animal, as determined by the epidemiologist designated by the District Director, an accredited or regulatory veterinarian must collect blood for an official brucellosis screening test of all test-eligible animals in the balance of the herd.
- If disease is confirmed, the herd will be considered affected and managed accordingly. (See [Brucellosis or bovine tuberculosis affected herd management standards](#).)

- If all test-eligible animals are negative to the screening test, then the herd may be released from movement restrictions.
- A secondary (corroboratory) test must be conducted on all animals with a non-negative response to the screening test. All animals with a non-negative response to the secondary test will be classified by the epidemiologist designated by the District Director and subjected to further testing if deemed necessary by the epidemiologist designated by the District Director to determine their disease status.
- If there is no evidence of disease found after testing all remaining test eligible animals the herd may be released from movement restrictions.
- If it is not possible to identify the diagnostic exposed animals or if no diagnostic exposed animals are still present in the herd, follow the testing protocol as indicated in Epidemiological investigation testing for brucellosis.

Figure 3 Brucellosis testing of diagnostic exposed animals



4.1.6.2 Animals for diagnostic purposes: bovine tuberculosis

Indemnification is preferred to testing because it enables a rapid and accurate diagnosis and eliminates potential spread of bovine tuberculosis to susceptible animals in the receiving herd or calf raiser. All indemnified exposed animals must be submitted for postmortem examination. If disease is confirmed, the herd will be considered affected and managed accordingly. See [Brucellosis or bovine tuberculosis affected herd management standards](#). If there is no evidence of disease found during postmortem examination, a regulatory veterinarian must conduct an official test of all remaining test-eligible animals in the herd. See Epidemiological investigation testing for bovine tuberculosis.

If indemnification of identified exposed animals for diagnostic purposes is not possible, then a regulatory veterinarian must conduct an official test on the identified exposed animals with the cervical tuberculin (CT) test. Do not use the CFT test in cattle or bison, single cervical test (SCT) in captive cervids, comparative cervical tuberculin (CCT) test, or gamma interferon test for tuberculosis as the screening test on exposed animals.

- If the CT test is negative, a regulatory veterinarian must conduct an official test of all remaining test-eligible animals in the herd. See Epidemiological investigation testing for bovine tuberculosis. All exposed animals classified as negative to the initial CT test must remain under movement restrictions until they have completed a testing protocol in accordance with a herd plan developed for their disease affected herd of origin as indicated in [Brucellosis or bovine tuberculosis affected herd management standards](#).
- Any animal exhibiting a response to the CT test must be submitted for postmortem evaluation.
 - If disease is confirmed, the herd will be considered affected and managed accordingly. See [Brucellosis or bovine tuberculosis affected herd management standards](#).
 - If there is no evidence of disease found after postmortem examination, a regulatory veterinarian must conduct an official test of all remaining test eligible animals in the herd. See Epidemiological investigation testing for bovine tuberculosis.

4.1.7 Feedlots

Except for movement to other quarantine feedlots or pens, program animals that reside in feedlots and that are known to be exposed to brucellosis or bovine tuberculosis shall be restricted to movement directly to slaughter and shipped under permit. Feedlots or portions of feedlots that have contained brucellosis- or bovine tuberculosis-infected and exposed program animals shall be vacated, cleaned, and disinfected following the removal of the animals for slaughter (see [Appendix 10: Cleaning and Disinfection](#)).

Restocking a feedlot or portion of a feedlot that has contained brucellosis- or bovine tuberculosis-infected or -exposed program animals shall not begin until the required cleaning and disinfection have been completed and the feedlot or portion of the feedlot that contained infected program animals has been vacant of all livestock for a for a minimum of 30 days.

Quarantine feedlots and quarantine pens are exempt from the requirement to hold pens vacant for 30 days following the required cleaning and disinfection.

4.1.8 Wildlife

It is recommended, but not required, that disease surveillance in wildlife is conducted in areas where brucellosis or bovine tuberculosis has been detected in a dairy, beef, bison, or captive cervid herd and is not previously known to be present in wildlife. The main objective of the surveillance strategies described is to determine whether brucellosis or tuberculosis is present in wildlife surrounding infected premises. The Guidelines for Surveillance of Bovine Tuberculosis in Wildlife, located at http://www.aphis.usda.gov/animal_health/animal_diseases/tuberculosis/, should be used as a reference document concerning such surveillance.

Interagency cooperation is strongly encouraged between State or Tribal wildlife agencies, other State or Tribal cooperators, stakeholders, APHIS' Wildlife Services (WS), and VS to develop an effective area-specific surveillance plan. Additionally, coordination with NVSL before sampling is recommended to ensure timely testing of samples.

4.1.9 Non-program animal investigations

Animal species other than cattle, bison, and captive cervids may serve as reservoirs of brucellosis or bovine tuberculosis. These reservoirs may hinder disease eradication efforts. The epidemiological investigation of an affected herd should include careful evaluation of these other susceptible species of animals. Particular attention must be paid to those species exposed to *B. abortus* or *M. bovis*. If any herds of other species of domestic livestock have been found to be affected with brucellosis or bovine tuberculosis, they must be tested and found negative, slaughtered, or quarantined so that no foci of brucellosis or bovine tuberculosis in any species of domestic livestock are left uncontrolled. A brief guide for testing some of these species follows.

4.1.9.1 Poultry

Although not susceptible to *M. bovis*, poultry infected with *M. avium* may sensitize or infect cattle or bison.

- Flocks should be inspected for evidence of clinical disease.
- Some birds should be necropsied to detect lesions of tuberculosis. The thinnest birds in the flock should be selected for this purpose.

- The essentials of proper management for maintenance of a tuberculosis-free flock should be discussed with the owner.
- Chickens should be tested by injecting 0.05 ml purified protein derivative (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.

4.1.9.2 Swine

- Brucellosis: Swine should be tested using an official test for brucellosis. Blood should be drawn and submitted to an approved laboratory for official testing. Consultation with NVSL is recommended for guidance on appropriate serologic tests for swine.
 - Regardless of whether program animals are depopulated or are maintained under quarantine on an affected premises for *B. abortus*, swine on brucellosis-affected premises should be appropriately tested for exposure to brucellosis. Brucellosis-affected premises and surrounding areas should be evaluated for the presence of feral swine. Feral swine infected with *B. suis* may expose and infect cattle or bison with *B. suis*. If exposed, swine can become infected with *B. abortus*.
- Bovine tuberculosis: Swine should be tested using an intradermal injection of 0.1 ml each of PPD bovine and/or PPD avian in the skin on the back of the ear at the lateral edge near the base (alternatively, the vulva of sows or anal ring of boars). Readings should occur at 48 hours after injection by observing and palpating for swelling.
 - The possibility of spread of *M. bovis* between cattle, bison, and swine should always be investigated. If non-swine program animals are depopulated on an affected premises for *M. bovis*, swine on the affected premises should be sent to slaughter.

4.1.9.3 Goats

- Brucellosis: Goats should be tested for brucellosis. Blood should be drawn and submitted to an approved laboratory for testing. Consultation with NVSL is recommended for guidance on appropriate serologic tests for goats.
- Bovine tuberculosis: The same tuberculin testing procedures as for cattle and bison should be followed. The age for tuberculin testing goats is 6 months and over. Owners should be advised that an accredited herd plan for *M. bovis* for dairy goats is available (see [Appendix 11: Brucellosis or Bovine Tuberculosis Accredited Goat Herds](#))

4.1.9.4 *Sheep*

- Brucellosis: Sheep should be tested for brucellosis. Blood should be drawn and submitted to an approved laboratory for testing. Consultation with NVSL is recommended for guidance on appropriate serologic tests for sheep.
- Bovine tuberculosis: Follow the same tuberculin testing procedures as for cattle and bison. Sheep on affected premises depopulated for *M. bovis* should be sent to slaughter.

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4.1.9.5 *Cats, dogs, and horses*

Companion animals are susceptible to brucellosis and bovine tuberculosis and may become a source of infection to a new herd. It is recommended that they not be housed with program animals or stored feed.

- Brucellosis: If the epidemiologist designated by the District Director deems it appropriate, working dogs and horses should be tested for brucellosis. Consultation with NVSL is recommended for guidance on appropriate serologic tests for on-farm companion animal species such as horses and dogs.
- Bovine tuberculosis: Working dogs and horses may be used to work program animals.

4.1.9.6 *Camelidae (camel, llama, alpaca, & guanaco)*

- Brucellosis: If deemed appropriate by the epidemiologist designated by the District Director, Camelidae should be tested for brucellosis. Blood should be drawn and submitted to an approved laboratory for testing. Consultation with NVSL is recommended for guidance on appropriate serologic tests for camelidae species.
- Bovine tuberculosis: 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 tuberculosis 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.

4.1.9.8 *Nonhuman primates*

- Brucellosis: If the epidemiologic investigation of a brucellosis-affected premises indicates possible exposure of brucellosis to nonhuman primates, the epidemiologist designated by the District Director, who will notify CHG specialists of this possible exposure. The epidemiologic investigation should include consultation with NVSL and Centers for Disease Control and Prevention (CDC) to determine appropriate follow-up guidelines and recommendations.
- Bovine tuberculosis: There are various testing procedures for nonhuman primates. The National Institute of Health (NIH) recommends intradermal testing with 0.1 ml of Old Tuberculin. Injections should be 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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4.1.9.7 Zoonosis

Humans may be affected by *Brucella abortus* or *Mycobacterium bovis*. It is important that people who work with affected herds be evaluated by a physician for these infections. Physicians or public health departments should be consulted regarding questions about testing humans.

The AD will notify or ensure that State or Tribal animal health officials notify State or Tribal public health authorities of brucellosis or bovine tuberculosis in livestock when the disease infection is confirmed in a herd. The AD will request that State or Tribal public health authorities notify the AD of brucellosis or bovine tuberculosis in humans when the disease infection is confirmed in humans.

4.1.10 Reporting

4.1.10.1 Affected herds

- An initial epidemiological investigation situation report will be completed by the epidemiologist designated by the District Director and submitted to CHG specialists within 15 days of identification of a herd as affected.
- Updated epidemiological investigation situation reports will be submitted to CHG specialists at least every 4 weeks following submission of an initial epidemiological situation report.
- Investigation closure (“closing”) reports will be submitted to CHG specialists within 60 days following conclusion of an epidemiological investigation.

4.1.10.2 Slaughter traces, trace outs and trace ins

- An initial investigation report will be completed by the epidemiologist designated by the District Director and submitted to CHG specialists within 15 days of initiation of an investigation for non-negative test results for brucellosis or *M. bovis*, or receipt of notification of an interstate trace.
- Updated epidemiological investigation situation reports will be submitted to CHG specialists at least every 4 weeks following submission of an initial report.
- Investigation closure reports will be submitted to CHG specialists within 60 days following conclusion of an epidemiological investigation.

4.2 Brucellosis or bovine tuberculosis affected herd management standards

4.2.1 Affected herd determination

VS will determine if a herd of program animals is brucellosis- or bovine tuberculosis -affected only after consulting with the State or Tribal Animal Health Officials of the State or Tribe in which the herd resides. VS will base this determination on available laboratory and epidemiological information and input from CHG, the appropriate CHG specialist, the appropriate epidemiologist designated by the District Director, and the appropriate AD.

Notification of a disease affected herd will be by publication on the VS Web site within 3 business days of determination of disease affected herd status.

The epidemiologist designated by the District Director will be responsible for completing and submitting to the CHG specialist the initial situation report within 15 days of determination of disease affected herd status.

4.2.1.1 Affected herd determination for brucellosis

The determination of affected herd status requires laboratory confirmation of *Brucellosis abortus* or substantial evidence of *B. abortus* infection such that the epidemiologist designated by the District Director determines a program animal from the herd is infected. This evidence generally consists of bacterial identification of *B. abortus* or official test results.

4.2.1.2 Affected herd determination for bovine tuberculosis

The determination of affected herd status requires laboratory confirmation of *Mycobacterium bovis* or substantial evidence of *M. bovis* infection such that the epidemiologist designated by the District Director determines a program animal from the herd is infected. This evidence would generally consist of bacterial identification of *M. bovis* or a polymerase chain reaction (PCR) test positive for *Mycobacterium tuberculosis* complex. Evidence may also include histopathology-compatible lesions in a known tuberculosis-exposed bovine.

4.2.2 Support of depopulation or test-and-remove herd management

VS will determine if a test-and-remove or depopulation management plan will be supported with Federal indemnity for each brucellosis- or bovine tuberculosis-affected herd of program animals based upon each herd's unique circumstances.

Immediately after a herd is classified as brucellosis- or bovine tuberculosis-affected, the CHG specialist will coordinate with the epidemiologist designated by the District Director, the AD, and the SAHOs to obtain the essential data regarding the affected herd, including the initial apparent disease prevalence within the herd. For brucellosis- or bovine tuberculosis-affected herds determined through the discovery of infection in exposed animals, a test of all test-eligible animals in the herd will be required to establish the initial apparent disease prevalence within that herd. Otherwise, the test of the herd that disclosed the infection will be used to determine the initial apparent disease prevalence.

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Evaluation criteria include, but are not limited to:

- The apparent prevalence of infection in the herd
- The risk of disease transmission while under a test-and-removal plan
- Effectiveness of other management practices to mitigate disease spread
- The cost effectiveness of depopulation

Notification of VS' support of a herd management protocol will be by publication on the VS Web site within 10 days of determination.

4.2.3 Affected herd management plans

A written affected herd management plan will be developed for all disease-affected herds of program animals. The epidemiologist designated by the District Director, AD, and SAHO will jointly develop the plan for the CHG specialist's and CHG's general approval.

- All herd management plans will include:
 - Provisions for complete herd inventory and regular inventory reconciliations.
 - Description and location(s) of premises involved.
 - Conditions for release of movement restrictions.
 - Biosecurity and disease transmission mitigation measures.
 - Replacement stock purchases.
 - Replacement stock testing schedules.
 - Any other items deemed necessary by the ep and the owner.
- Depopulation affected herd management plans will also include:
 - Method and schedule for depopulation.
 - Cleaning and disinfection. (See [Appendix 10: Cleaning and Disinfection.](#))
 - Any other items deemed necessary by the epidemiologist designated by the District Director and the owner.
- Test-and-remove affected herd management plans will also include:
 - Animals to be tested.
 - Tests and laboratories to be used.
 - Testing schedules.
 - Test interpretation and classification of animals.
 - Disposition of animals based on classification.
 - Requirements and destinations for movements of animals.
 - Assurance testing.
 - Any other items deemed necessary by the epidemiologist designated by the District Director and the owner.

4.2.4 Movements from brucellosis or bovine tuberculosis affected herds

Movements of animals from brucellosis- or bovine tuberculosis-affected herds will be permitted only under the following conditions:

- Between premises identified in the herd management plan.
- Directly from the affected premises to a recognized slaughtering establishment under permit.
- Directly from the affected premises to necropsy under permit.
- Directly to a quarantine feedlot, quarantine pen, or a previously documented terminal operation, if the animals to be moved are negative to a screening test and if they are sexually intact and 6 months of age for brucellosis or over 2 months of age for bovine tuberculosis.

4.2.5 Test and remove protocol

- The testing portion of a test-and-removal protocol will consist of three stages: Removal testing, verification testing, and assurance testing. The herd management plan will specify what tests will be used and the intervals between all tests.
- The interval between any two consecutive tests will be calculated as:
 - For brucellosis from bleed date to bleed date.
 - For bovine tuberculosis from injection date to injection date.
- During the test-and-removal protocol, if investigators find evidence, including any evidence from slaughter surveillance, that any animal in the herd is infected, investigators will update any herd testing model simulations as appropriate, re-evaluate both the herd specific test-and-remove protocol and the herd management recommendations, and return the herd to the first test of the removal testing stage. The animal must be linked conclusively with the affected herd, e.g., through official identification, trace-back evidence, etc.
- To release a herd from quarantine, removal and verification testing must be completed without evidence of infection.
 - For brucellosis:
 - 1 negative removal test conducted 30–60 days after the last reactor has been removed and slaughtered.
 - 1 verification test conducted 6 months after the negative removal test.
 - For bovine tuberculosis:
 - 2 negative removal tests are required and the second removal test must achieve a 95 percent or greater confidence level that the herd is bovine tuberculosis-free before conducting the verification test.

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- 1 verification test conducted no sooner than 180 days after the last removal test.
- When these criteria and all other provisions of the herd management plan have been fulfilled, the movement restrictions may be released and assurance testing begun.

4.2.5.1 Removal testing

- The purpose is to remove any animals that are sensitized to *Brucella* or *Mycobacterium* antigens and that may be infected. All test-eligible animals will be tested with the screening test.
- Animals may also be tested with other approved tests as determined by the epidemiologist designated by the District Director and described in the affected herd management plan. If multiple tests are used during removal testing, these tests and their interpretation will be defined in the test-and-remove herd plan.
- An accredited or regulatory veterinarian may draw blood for removal testing of brucellosis affected herds. A regulatory veterinarian must conduct all removal testing in a bovine tuberculosis affected herd.
- If disease is identified during any test in the removal stage, the herd must be returned to the first removal test of the test-and-remove protocol.
- Test-eligible age for removal testing:
 - Brucellosis – 6 months and older
 - Bovine tuberculosis – 2 months and older
- Removal test intervals
 - Brucellosis – 30 days between blood draws.
 - Bovine tuberculosis – 60 days between any 2 intradermal PPD injections.
- Brucellosis:
 - If all test-eligible animals are negative to the screening test, the herd may advance to the next test in the test-and-remove protocol.
 - Further testing may be conducted on all animals with a nonnegative response to the screening test as described in the affected herd management plan. All animals with a nonnegative response to further testing will be classified by the epidemiologist designated by the District Director or the CHG specialist and evaluated as deemed necessary by the epidemiologist designated by the District Director to determine their disease status.
 - One negative removal test conducted 30–60 days after the last reactor has been removed and slaughtered is required before conducting the verification tests.
- Bovine tuberculosis:

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- Slaughter all animals that are non-negative to the screening test and submit tissues from all such animals to NVSL. If there is no evidence of bovine tuberculosis, the herd may advance to the next test in the test-and-remove protocol.
- Two negative removal tests are required and the second removal test must achieve a 95 percent or greater confidence level that the herd is bovine tuberculosis-free before conducting the verification test.

4.2.5.2 Verification testing

- The purpose is to assure as much as possible that newly developed sensitivity to *Brucella* or *Mycobacterium* in the herd is not related to disease. The secondary (“corroboratory”) test or other approved tests as determined by the epidemiologist designated by the District Director and described in the affected herd management plan may be used on animals that are non-negative on the screening test to differentiate sensitivity due to *B. abortus* or *M. bovis* from other inconsequential bacteria.
- Animals classified as brucellosis suspect or reactor by the secondary test must be further evaluated as determined by the epidemiologist designated by the District Director. Animals classified as bovine tuberculosis suspect or reactor by the secondary test must be examined postmortem for evidence of disease.
- An accredited or regulatory veterinarian may draw blood for removal testing of brucellosis affected herds. A regulatory veterinarian must conduct all removal testing in a bovine tuberculosis-affected herd.
- If disease is identified during the verification test, the herd must be returned to the first removal test of the test-and-remove protocol.
- Verification tests may be applied no sooner than:
 - Brucellosis – 6 months after the negative removal test
 - Bovine tuberculosis – 180 days after the last removal test
- Test eligible age for verification testing for:
 - Brucellosis – 6 months and older
 - Bovine tuberculosis – 6 months and older
- All animals above the recommended minimum age plus any non-natural additions regardless of age will be tested with the screening test. Animals that respond to the screening test may be tested with a secondary test. Animals may also be tested with other approved tests as appropriate as described in the affected herd management plan.
- Brucellosis:
 - If all test eligible animals are negative to the screening test, the herd may be released from movement restrictions and advanced to the assurance testing stage.
 - Further testing may be conducted on all animals with a nonnegative response to the screening test as described in the affected herd management plan. All animals with a

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- nonnegative response to further testing will be classified by the epidemiologist designated by the District Director or CHG specialist and evaluated as deemed necessary by the epidemiologist designated by the District Director to determine their disease status.
- If after further evaluation there is no evidence of brucellosis the herd may be released from movement restrictions and advanced to the assurance testing stage.
 - Bovine tuberculosis:
 - If all test-eligible animals are negative to the screening test, the herd may be released from movement restrictions and advanced to the assurance testing stage.
 - All animals with a nonnegative response to the screening test must be tested with a secondary test as described in the affected herd management plan.
 - If all animals are negative to the secondary test, then the herd may be released from movement restrictions and advanced to the assurance testing stage.
 - Animals classified as suspect to the secondary test may be retested after the appropriate time interval. See [Laboratory and Diagnostic Test Approval](#) for a description of appropriate intervals.
 - Animals classified as reactor to the secondary test must be submitted for necropsy. If there is no evidence of bovine tuberculosis, the herd may be released from movement restrictions and advanced to the assurance testing stage.
 - All herd plans must include provisions for assurance testing as described below as a condition of quarantine release.

4.2.5.3 Assurance testing

- The purpose is to address the small estimated risk that a previously affected herd silently maintains disease infection.
- The extended testing period allows any infection that might still be present and undetected to spread to other susceptible individuals within the herd and be detected. This testing addresses the possible reintroduction of disease through imported livestock, infected wildlife, or other sources of infection.
- Assurance testing requirements after release of movement restrictions, as determined by the epidemiologist designated by the District Director and CHG specialist, will be specified in the affected herd management plan and will be included as a provision for release of movement restrictions after successful completion of verification testing.
- Animals classified as brucellosis suspect or reactor by the secondary test must be further evaluated as determined by the epidemiologist designated by the District Director.
- Animals classified as bovine tuberculosis suspect to the secondary test may be retested after the appropriate time interval. See [Laboratory and Diagnostic Test Approval](#) for a description of appropriate intervals.

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- Animals classified as bovine tuberculosis reactor to the secondary test must be submitted for necropsy. If there is no evidence of bovine tuberculosis, the herd may be advanced to the next test in the test-and-remove protocol.
- An accredited or regulatory veterinarian may draw blood for assurance testing of brucellosis affected herds. A regulatory veterinarian must conduct all assurance testing in a bovine tuberculosis affected herd.
- If disease is identified during assurance testing, the herd must be returned to the first removal test of the test-and-remove protocol.
- Following the release of quarantine, the first assurance test may be applied no sooner than:
 - Brucellosis – 1 year after verification test
 - Bovine tuberculosis – 1 year after the verification test
- Number of tests and time period for completion of testing:
 - For brucellosis – one test of all test-eligible animals in the herd within two years after release of movement restrictions.
 - For bovine tuberculosis – three to five tests of all test-eligible animals in the herd within five years after release of quarantine. The final assurance test must be conducted between 4 and 5 years after release of quarantine.
- Test-eligible age for assurance testing for:
 - Brucellosis – 6 months and older.
 - Bovine tuberculosis – 12 months and older.
- All animals above the recommended minimum age plus any non-natural additions regardless of age will be tested with the screening test. Animals that respond to the screening test may be tested with a secondary test. Animals may also be tested with other approved tests as appropriate and described in the affected herd management plan.
- Brucellosis:
 - If all test-eligible animals are negative to the screening test, the assurance stage of the affected herd management plan is completed.
 - Further testing may be conducted on all animals with a nonnegative response to the screening test as described in the affected herd management plan. All animals with a nonnegative response to further testing will be classified by the epidemiologist designated by the District Director or CHG specialist and evaluated as deemed necessary by the epidemiologist designated by the District Director to determine their disease status.
 - If after further evaluation there is no evidence of brucellosis, the assurance stage of the affected herd management plan is completed.

- Bovine tuberculosis:
 - If all test-eligible animals are negative to the screening test, the herd may advance to the next test in the test-and-remove protocol.
 - When the number of assurance tests specified in the herd plan has been completed with negative results, the affected herd management plan is completed.
 - All animals with a nonnegative response to the screening test must be tested with a secondary test as described in the affected herd management plan.
 - If all animals are negative to the secondary test, then the herd may be advance to the next test in the test-and-remove protocol.
 - Animals classified as suspect to the secondary test may be retested after the appropriate time interval. See Laboratory and Diagnostic Test Approval for a description of appropriate intervals.
 - Animals classified as reactor to the secondary test must be submitted for necropsy. If there is no evidence of bovine tuberculosis, the herd may be advance to the next test in the test-and-remove protocol.

4.2.6 Procedures in brucellosis- or bovine tuberculosis-affected feedlots

- A brucellosis- or bovine tuberculosis-affected feedlot shall be handled in the same manner as an affected herd in regard to epidemiological investigation and the development of epidemiological tracings for animal movements into and out of the feedlot. Emphasis during epidemiological investigations shall be on detecting possible spread from the feedlot.
- Unless previously documented biosecurity measures are in place, all animals in the feedlot will be considered exposed and movements restricted to the same destinations as for an affected herd until all exposed animals have been removed from the feedlot. Segregated animals may move to destinations other than slaughter following a negative screening test.

4.2.7 Procedures in brucellosis-or bovine tuberculosis-affected calf raising facilities

- A brucellosis or bovine tuberculosis affected calf raising facility shall be handled in the same manner as an affected herd in regard to epidemiological investigation and the development of epidemiological tracings for animal movements into and out of the facility. Emphasis during epidemiological investigations shall be on detecting possible spread from the facility.
- Unless previously documented biosecurity measures are in place all animals in the facility will be considered exposed and movements restricted to slaughter only channels until all exposed animals have been removed from the premises. Segregated animals may move to destinations other than slaughter channels following a negative screening test.

Element 5: Indemnity

APHIS will develop a separate comprehensive regulation and Program Standards document that addresses indemnity in multiple disease programs and species including swine, cattle, cervids, and animals depopulated as a result of a declared emergency (i.e., a foreign animal disease).

Until these changes are made, several existing memoranda describe policies and procedures for indemnity associated with the brucellosis and bovine tuberculosis programs. These include:

- VS Memorandum 534.1, Compensation: Procedures, Appraisal, and Indemnity Claim
- VS Memorandum 551.29, Indemnity Claims for Brucellosis
- VS Memorandum 552.32, Indemnity Claims for Tuberculosis

Element 6: Interstate Movement Requirements

Regulatory authority

- 9 CFR 76.8 through § 76.17, Interstate movement requirements
- 9 CFR 71.3, Interstate movement of diseased animals and poultry generally prohibited.
- 9 CFR 71.17, Interstate movement of dead poultry or other animals prohibited in same car with live poultry or other animals.
- 9 CFR 86.4, Official identification.
- 9 CFR 86.5, Documentation requirements for interstate movement.

Background

Movement controls are activities intended to reduce the potential for transmission and mitigate risk of these infections. They prevent the spread of the infection from areas of identified risk (such as quarantined facilities or established recognized management areas where risk is documented or when there is failure to meet program requirements). Movement controls will apply to:

- Identified areas, such as quarantined facilities or recognized management areas; but may also apply to a risk population without regard to program status in a State, Tribe, or recognized management area.
- Any State or Tribe that does not comply with or otherwise lapses from Federal, State, or Tribal standards for investigation activities, surveillance, or affected herd management, among other activities.
- Animals that originate from a known or suspect risk area or population.

The regulations in 9 CFR 76.8 through § 76.17 provide:

- Interstate movement controls for animals presenting a risk of spread of brucellosis or bovine tuberculosis.
- The types and classes of animals and herds that are subject to movement controls.

- Consequences for lack of implementation, maintenance, and compliance with risk mitigation measures or restrictions.
- That the Administrator can, in specific cases, consider conditions for variances.

6.1 Interstate movement requirements applicable to all classes of animals from all State and Tribe statuses

Any and all animals moving interstate must:

- Have official identification, with certain limited exceptions (see 9 CFR 86.4);
- Be accompanied by an interstate certificate of veterinary inspection (ICVI), with certain limited exceptions (see 9 CFR 86.5); and
- Not be moved in the same car within a means of conveyance as dead poultry or other dead livestock (see 9 CFR 71.17).

6.2 Interstate movement prohibitions

Interstate movement of the following classes of livestock or program animals is generally prohibited unless otherwise noted:

- Any livestock known to be infected with brucellosis or bovine tuberculosis
 - Except that the Administrator may provide for the interstate movement of individual animals under such conditions as he or she prescribes to prevent the spread of infection (9 CFR 71.3(d)(7)).
- Any program animals from a herd containing a suspect or reactor for bovine tuberculosis, other than the suspect or reactor itself, until the disease status of all test-eligible animals in the herd is determined.

6.3 Interstate movement of reactor, suspect, and exposed program animals

Program animals that have been classified as brucellosis or bovine tuberculosis reactors, suspects, or exposed animals may be moved interstate provided the animals:

- Are officially identified.
- Are accompanied by a completed VS Form 1027, Permit for Movement of Restricted Animals, which specifies the classification of the animals.

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- Are moved for diagnostic testing, immediate slaughter, necropsy or other use as approved by the Administrator.
- Are moved to a destination approved by the Administrator.
 - Approved destinations for reactors, suspects, and exposed animals are:
 - A recognized slaughtering establishment.
 - A diagnostic laboratory approved by the Administrator.
 - A research facility.
 - A specifically approved stockyard.
 - Any other destination specified within an APHIS-approved animal health plan.
 - Additionally, exposed animals that have tested negative to a screening test for brucellosis or bovine tuberculosis may be moved to quarantine feedlot or quarantine pen.
- In addition, the conveyance in which the animals are moved must:
 - Contain only animals not susceptible to brucellosis and bovine tuberculosis or animals destined for immediate slaughter or necropsy.
 - Be secured with official seals applied and removed by an authorized APHIS representative, State or Tribal representative, FSIS inspector, accredited veterinarian, or other individual authorized for this purpose by an APHIS representative. (Please note: Instead of this requirement, the animals may be accompanied during movement by an APHIS representative, FSIS inspector, State or Tribal representative, or other individual authorized for this purpose by an APHIS representative.)
 - Be cleaned and disinfected after shipment of the animals by the carrier in accordance with 9 CFR 71, under the supervision of an APHIS representative, FSIS inspector, State or Tribal representative, accredited veterinarian, or other person designated by the Administrator.

6.4 Consistent States and Tribes

- *Rodeo, event, or exhibited cattle or bison may be moved interstate provided that they:*
 - Test negative to an individual official test for bovine tuberculosis no more than 60 days prior to their initial movement from the premises of origin.
 - Test negative to an individual official test for bovine tuberculosis no more than 180 days prior to any subsequent interstate movement.
 - Test negative to an individual official test for brucellosis no more than 60 days prior to their initial movement from the premises of origin if they are sexually intact and 6 months of age or older.
 - Test negative to an individual official test for brucellosis no more than 180 days prior to any subsequent interstate movement, if they are sexually intact and 6 months of age or older.
 - Are accompanied during interstate movement by an interstate certificate of veterinary inspection (ICVI) stating the date, location, and test results for the testing administered prior to their initial interstate movement and for the last official test administered.
 - Are officially identified.

- *Movement of all other cattle or bison:*
 - From all areas other than a recognized management area:
 - Must move in accordance with the general movement requirements in Section [6.1](#), but have no further restrictions.
 - From a recognized management area for brucellosis or bovine tuberculosis:
 - May only be moved in accordance with the conditions for movement of program animals from such a recognized management area as specified in the State's or Tribe's animal health plan.

6.5 Provisionally consistent States or Tribes

- Unless specified otherwise in the *Federal Register* notice designating the State or Tribe as a provisionally consistent State or Tribe, cattle or bison moved interstate from any area within a provisionally consistent State or Tribe are subject to the conditions for movement of cattle or bison from a consistent State or Tribe.
- If the notice in the *Federal Register* designating the State or Tribe as a provisionally consistent State or Tribe specifies restrictions on the interstate movement of cattle or bison from the State, and these restrictions differ from the conditions for interstate movement of cattle or bison from a consistent State or Tribe, the interstate movement of such cattle or bison is subject to the restrictions specified in the notice in the *Federal Register*.

6.6 Inconsistent States or Tribes for brucellosis

- Sexually intact cattle or bison that are six months or age or older may be moved interstate:
 - For immediate slaughter if they are officially identified and accompanied by an ICVI.
 - For purposes other than for immediate slaughter if:
 - They originate from a herd that has had a negative herd test for brucellosis no more than one year and no less than 120 days prior to movement.
 - The individual cattle or bison moving have had an additional negative individual test for brucellosis no more than 60 days prior to movement.
 - Since the individual test, the individual cattle or bison have not commingled with non-natural herd additions of unknown brucellosis status or animals that have had a non-negative test for brucellosis.
 - The cattle or bison are officially identified.
 - The cattle or bison are accompanied by an ICVI documenting both the negative herd test results and the negative individual animal test results.
- Cattle or bison less than six months of age, steers, and spayed heifers may move interstate provided they are officially identified and accompanied by an ICVI.

6.7 Inconsistent States or Tribes for bovine tuberculosis

Cattle and bison may be moved interstate:

- For immediate slaughter if they are officially identified and accompanied by an ICVI.
- For purposes other than for immediate slaughter provided:
 - They originate from a herd that has had a negative herd test for bovine tuberculosis no more than one year and no less than 120 days prior to movement.
 - The individual cattle or bison moving have had an additional negative individual test for bovine tuberculosis no more than 60 days prior to movement.
 - Since the individual test, the individual cattle or bison have not commingled with non-natural herd additions of unknown bovine tuberculosis status or animals that have had a non-negative test for bovine tuberculosis.
 - The cattle or bison are officially identified.
 - The cattle or bison are accompanied by an ICVI documenting both the negative herd test results and the negative individual animal test results.

6.8 Captive cervids

- Captive cervids originating directly from herds currently accredited for both brucellosis and bovine tuberculosis may be moved interstate provided that they are officially identified and accompanied by an ICVI which states that they originate directly from a herd currently accredited for both brucellosis and bovine tuberculosis.
- Captive cervids destined for immediate slaughter must:
 - Be officially identified.
 - Be accompanied by an ICVI.
 - Be moved directly to a recognized slaughtering establishment.
- If not destined for immediate slaughter or from a currently accredited herd, captive cervids must:
 - Originate from a herd that has had a negative herd test for both brucellosis and bovine tuberculosis no more than 1 year prior to movement.
 - Have had an additional negative individual test for both brucellosis and bovine tuberculosis no more than 60 days prior to movement.
 - Be officially identified.
 - Be accompanied by an ICVI.
 - If from an inconsistent State or Tribe for brucellosis or bovine tuberculosis, not have commingled with non-natural herd additions of unknown disease status or that have had a non-negative test for brucellosis or bovine tuberculosis.

6.9 Requirements for accredited herds

6.9.1 Initial accreditation and reaccreditation

- To qualify for accredited herd status, the herd must pass at least two consecutive official brucellosis herd blood tests and/or bovine tuberculosis tests conducted within an interval of 9-15 months with no evidence of or exposure to brucellosis or bovine TB.
- As an alternative, dairy cattle herds may qualify for brucellosis accredited herd status, by conducting a minimum of four consecutive negative brucellosis ring tests, or other official brucellosis milk test approved by the Administrator, at not less than 90-day intervals, followed by a negative herd blood test within 90 days after the last negative brucellosis ring test or other official brucellosis milk test approved by the Administrator.
 - Additions to the herd during the qualifying period for accreditation:
- Other than natural additions, any additions to the herd must meet the requirements specified in 6.9.4 Accredited herd additions.
 - When being tested for initial accreditation or reaccreditation, all program animals on the premises must have negative results, regardless of whether they are members of the herd.
 - Herds meeting the qualifications for accreditation must be issued a certificate of accreditation and other appropriate information must be provided by the local State and Federal animal health officials to emphasize the significance of herd accreditation.
 - Accreditation periods will be based on the anniversary date of accreditation and not on the dates of reaccreditation testing.
- For herds in consistent States or Tribes, the entire State or Tribe, or, if the State or Tribe has an RMA, the portion of the State or Tribe that is not the RMA:
 - For cattle and bison herds: The accreditation period will be 24 months from the anniversary date.
 - For captive cervid herds: The accreditation period will be 36 months from the anniversary date.
- For herds in a provisionally consistent State or Tribe, the entire State or Tribe, or, if the the State or Tribe has an RMA, the portion of a State or Tribe that is not the RMA:
 - For cattle and bison herds: The accreditation period will be 12 months from the anniversary date.
 - For captive cervid herds: The accreditation period will be 24 months from the anniversary date.
- Herds in inconsistent States or Tribes, or in RMAs:

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- Accredited herds will not be recognized in inconsistent States or Tribes, or in RMAs.
- All purchased additions must be recorded on the test report as purchased addition members of the herd at the time of the accreditation or reaccreditation herd test.
- To qualify for reaccreditation, the herd must pass a negative herd reaccreditation test within +/- 3 months of the herd's accreditation anniversary date.
- For brucellosis herd reaccreditation, an official brucellosis herd blood test must be conducted.
- As an alternative for dairy cattle herds, a minimum of four consecutive negative brucellosis ring tests must be conducted at approximately 90-day intervals, with the fourth test conducted within 60 days before the 1-year anniversary of the previous certification date.
- The Administrator may allow another testing protocol to be used if the Administrator determines that such a protocol is adequate to determine there is no evidence of brucellosis in the herd.
- For continuous herd accreditation, the reaccreditation herd test must be conducted on the anniversary date or within the 3 months preceding the anniversary date.
- If the reaccreditation herd test is conducted after the anniversary date, the accredited status of the herd will be suspended until the reaccreditation herd test is completed.
- If the reaccreditation herd test is not completed within 3 months after the anniversary date, the herd will lose accredited status and the requirements for reaccrediting the herd will be the same as for initial herd accreditation.

6.9.2 Herd inventory reconciliations

- At each herd test, either initial accreditation or reaccreditation, a complete inventory of all program animals in the herd, regardless of age, will be performed.
- All program animals in the herd not present on the premises at the previous herd test must be reconciled as to their origin.

6.9.3 Artificial insemination

Semen used for artificial insemination of animals in an accredited herd must be from sires in currently accredited herds or from sires with a negative result on an official test for brucellosis and bovine tuberculosis performed within 12 months prior to the date of the semen collection.

6.9.4 Accredited herd additions

- Animals other than natural additions added to an accredited herd will not receive the accredited herd status for sale or movement purposes until they have been included in a reaccreditation herd test.

- From herds in a consistent State or Tribe, the entire State or Tribe, or, if the State or Tribe has an RMA, the portion of the State or Tribe that is not the RMA:
 - From accredited herds: Animals may be moved directly from an accredited herd of origin and immediately commingled with the animals in the destination herd.
 - From herds not accredited: Animals originating from a non-accredited herd in a consistent State or Tribe must have a negative test for the disease of interest within 10 days of movement. Animals with a negative test for the disease of interest may be immediately commingled with the animals in the destination herd.
- From herds in a provisionally consistent State or Tribe, the entire State or Tribe, or if the State or Tribe has an RMA, the portion of the State or Tribe that is not the RMA:
 - From accredited herds: Animals originating from an accredited herd in a provisionally consistent State or Tribe must have a negative test for the disease of interest within 10 days of movement. Animals with a negative test for the disease of interest may be immediately commingled with the animals in the destination herd.
 - From herds not accredited: The herd of origin for the additions must have received a negative herd test within the past 12 months, and the individual animals for addition must have a negative test for brucellosis if they are sexually intact, 6 months of age and older and a negative test for bovine tuberculosis if they are 2 months of age and older, within 10 days prior to entering the premises of the accredited herd.
- From herds in an inconsistent State or Tribe: No additions to an accredited herd may originate from any herd in an inconsistent State or Tribe.
- Additions that originate from a herd within an RMA: If a program animal originates from a herd within an RMA, it must be moved to a non-accredited herd outside of the RMA and reside in that herd for at least four months before it may be added to an accredited herd. It will then be subject to the relevant requirements above for an addition from a non-accredited herd.

6.10 Requirements for APHIS approval of quarantine feedlots and quarantine pens

6.10.1 Quarantine feedlot/feedyard

- A quarantine feedlot/feedyard may be approved in accordance with the following requirements to house high risk program animals.
- A quarantine feedlot/feedyard can accept other program animals. These animals will be reclassified as high risk upon entry.
- Program animals entering and/or leaving a quarantine feedlot/feedyard must be officially identified, with traceability to the animal's most recent herd of residence.

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- Animals that die while in a quarantine feedlot/feedyard must be recorded in feedlot/feedyard records within 5 days of death. Records of deaths will include, at a minimum, all known identification, official or otherwise, and the date the animal was noted as dead.
- Requirements for necropsy and sampling of animals that die while in the feedlot/feedyard will be established by the epidemiologist designated by the District Director and the feedlot owner or designated representative.
- Animals born while in a quarantine feedlot/feedyard will be officially identified within 5 days of birth. The official identification applied, along with birth date, breed, and sex of the animal will be reported to regulatory authorities.
- A quarantine feedlot/feedyard will maintain records of all movements of animals into or out of the premises. These records must ensure traceability of any animal that is in, or has gone through, the quarantine feedlot/feedyard to its most recent herd of residence. These records must be available to regulatory personnel at any time during regular business hours or upon special request. All records must be maintained for a minimum of 5 years after the animal has left the quarantine feedlot.
- A quarantine feedlot/feedyard must be inspected before approval. Follow-up inspections must be conducted by regulatory personnel at 6 to 9 month intervals thereafter.
 - Items to be inspected include:
 - Biosecurity measures
 - Geographic separation
 - Condition of all fences
 - Wildlife interface risks
 - Availability of records
 - Accuracy of records
 - Official identification present on animals in the quarantined feedlot
 - Waste management
 - Reports of inspection results will be submitted to the epidemiologist designated by the District Director, who will review the inspection report and approve or disapprove the individual application for initial operation or continued operation as an approved quarantine feedlot/feedyard. The epidemiologist designated by the District Director will report the current status of approved quarantine feedlots/feedyards following each inspection to the CHG specialist.
- Quarantine feedlots/feedyards must not allow grazing or pasturing of animals.
- Quarantine feedlot/feedyards will be jointly approved by the Chief State or Tribal Animal Health Official and the APHIS Administrator for feeding restricted cattle or bison.

- All animals must move directly from the quarantine feedlot to slaughter or another approved quarantine feedlot/feedyard or approved quarantine pen with the prior permission of the State or Tribal animal health official.

6.10.2 Approved quarantine pen

Quarantine pens within a feedlot/feedyard may be approved to house high risk program animals. Program animals that enter areas of the feedlot other than the quarantine pen will not necessarily be reclassified as high risk animals; however, all animals that enter the quarantine pen itself will be reclassified as high risk. Quarantine pens are subject to the documentation and inspection requirements listed below, and all requirements, other than inspection requirements, listed above for quarantine feedlots:

- The feedlot/feedyard containing the pen is documented as being a terminal feedlot/feedyard; that is, no animals entering the premises are moved outside of slaughter channels.
- Approved quarantine pens must be inspected before approval. Follow-up inspections must be conducted by regulatory personnel at 6 to 9 month intervals thereafter. Items to be inspected include:
 - Biosecurity measures:
 - Geographic separation
 - Buffer zone separated by two fences and a distance of at least 30 feet must be maintained between approved quarantine pens and other pens at the feedlot/feedyard.
 - Drainage from approved quarantine pens cannot flow through other areas of the feedlot.
 - No shared watering or feeding troughs between approved quarantine pens and other pens at the feedlot/feedyard.
 - Separate hospital/sick pen facilities for high risk animals
 - Separate processing/receiving facilities for high risk cattle, or facilities must be cleaned and disinfected after use by high risk animals. Standard operating procedures that can verify this system is being followed must be implemented and documented.
 - Condition of all fences
 - Availability of records
 - Accuracy of records
 - Official identification present on animals in the quarantined pens
 - Waste management

Element 7: Importation Requirements

Regulatory authority: 9 CFR Part 93, Subpart D, Importation of Ruminants

Background

APHIS has established a regional classification system to address the risk that the importation of ruminants from foreign regions may present of disseminating brucellosis and bovine tuberculosis.

As part of this classification system, a representative of the competent veterinary authority of any country or countries may request that APHIS classify a region for brucellosis or bovine tuberculosis. Requests for classification or reclassification must be submitted to APHIS electronically or through the mail as provided at http://www.aphis.usda.gov/import_export/animals/live_animals.shtml.

In evaluating requests, APHIS will take into consideration both the brucellosis or bovine tuberculosis prevalence in the region, and whether the regions have a program for brucellosis or bovine tuberculosis that meets certain standards.

For bovine tuberculosis, the standards are:

- Effective veterinary control and oversight within the region.
- Tuberculosis is a notifiable disease within the region.
- The region has a program in place for tuberculosis that includes, at a minimum:
 - Epidemiological investigations following the discovery of any infected animals or affected herds, or any animals or herds that have had non-negative test results following a test for tuberculosis, and documentation of these investigations;
 - Management of affected herds in a manner designed to eradicate tuberculosis from those herds, and documentation regarding this management;
 - Regulatory controls on the movement of livestock into, within, and from the region that correspond to the risk of dissemination of tuberculosis associated with such movement; and
 - Access to, oversight of, and quality controls for diagnostic testing for tuberculosis within the region.
- The region has surveillance in place that is equivalent to or exceeds federal standards for surveillance within the United States.

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For brucellosis, the standards are:

- There is effective veterinary control and oversight within the region.
- Brucellosis is a notifiable disease within the region.
- The region has a program for brucellosis in place that includes, at a minimum:
 - Epidemiological investigations following the discovery of any infected animals or affected herds, or any animals or herds that have had non-negative test results following a test for brucellosis, and documentation of these investigations;
 - Management of affected herds in a manner designed to eradicate brucellosis from those herds, and documentation regarding this management;
 - Regulatory controls on the movement of livestock into, within, and from the region that correspond to the risk of dissemination of brucellosis associated with such movement; and
 - Access to, oversight of, and quality controls on diagnostic testing for brucellosis within the region.
- The region has surveillance in place that is equivalent to or exceeds federal standards for brucellosis surveillance within the United States.
- If the region vaccinates for brucellosis, it is in a manner that has been approved by APHIS.

Element 8: Laboratory and Diagnostic Test Approval

Regulatory authority: 9 CFR 76.19, official tests, official testing laboratory

Background

To ensure that diagnostic tests meet the requirements for brucellosis or tuberculosis program use, VS will review and provide official approval of any brucellosis or tuberculosis test prior to its use in either program. For diagnostic kits, licensure with Center for Veterinary Biologics (CVB) will also be required to ensure that the biologics available for diagnosing disease are effective.

Any testing laboratory must be approved prior to conducting official program diagnostic testing in order to insure that the laboratory meets certain requirements.

Whenever possible, use of Mobile Information Management (MIM) technology is recommended for completing the VS forms described in this element.

8.1 Administrative procedures for diagnostic test and laboratory approval

8.1.1 Evaluation of tests proposed for official use in the brucellosis and bovine tuberculosis eradication programs

The Surveillance, Preparedness, and Response Services (SPRS) CHG specialists will evaluate diagnostic tests for official brucellosis or bovine tuberculosis program use in consultation with CVB, VS District Offices, NVSL, the Centers for Epidemiology and Animal Health (CEAH), and the Agricultural Research Services (ARS). This evaluation will consider guidelines provided by the U.S. Animal Health Association (USAHA), such as "Criteria for Evaluating Experimental Tuberculosis Test Performance for Official Test Status," which can be found on the Web at <http://www.usaha.org/Portals/6/Committees/tuberculosis/AppendixE-BOVINE TUBERCULOSISReport.pdf>.

Diagnostic test kits require licensure by CVB. [APHIS Directive 6901.1](#) "Diagnostic Test Kit – Licensing and Approval Procedures" provides an overview of this process.

8.1.1.1 Licensing requirements

- Prior to evaluation for official use in the brucellosis or bovine tuberculosis eradication program, test manufacturers will:
 - Apply to CVB for a product license.
 - Obtain a license from CVB.

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- Biologic products intended for use in species in which brucellosis or bovine tuberculosis is rare or not known to occur and for which there are insufficient data may be eligible for a conditional license if there is a reasonable expectation of sensitivity and specificity.
- A test kit licensed by CVB meets the objective of having sufficient diagnostic sensitivity and diagnostic specificity for the test's intended purpose.

8.1.1.2 Protocol for Veterinary Services field evaluation of tests

- The brucellosis or bovine tuberculosis staff will evaluate requests for licensed tests to be considered for official program use. For tests that merit evaluation, manufacturers will give CHG staff the data submitted to CVB to support product licensure. CHG staff will review the data and CVB's evaluation and provide written recommendations to the test manufacturer regarding field studies that will be required to evaluate the test for official program use. These recommendations will:
 - Specify the electronic collection format and data storage location. Existing electronic data capture and storage tools, such as Mobile Information Management Systems, will be used whenever possible.
 - Describe the responsibilities of each participating APHIS-approved laboratory.
 - Specify that sample collection to evaluate new tests will occur when official brucellosis or bovine tuberculosis tests are administered. Samples will be collected under the supervision of State or Federal regulatory animal health officials.
 - Specify that only approved veterinary diagnostic laboratories will administer tests during the field evaluation.
- Test manufacturers will propose a field study protocol that includes the requirements described below for VS approval. Field studies should be done in collaboration with CVB, CHG staff, VS District Offices, NVSL, CEAH, State animal health authorities, and APHIS-approved veterinary diagnostic laboratories. The State animal health official must approve the field study in all States where studies will be conducted.
- Factors to address in field study protocol:
 - State the intended use(s) of the test in the brucellosis or bovine tuberculosis program.
 - State the specific objectives(s) of the study/field trial. For instance, is the field study a side-by-side blind comparison of the new test or a field trial? State sample size and justify this number (i.e., estimated sensitivity and specificity of test under evaluation, margin of error/power, etc.).
 - Describe the proposed study.
 - Describe the source of samples to be tested:
 - Herd selection, number of herds, demographics of herds (geographic location, production type/breeds, brucellosis or bovine tuberculosis-exposure status, etc.)
 - Individual animal selection
 - Inclusion and exclusion criteria for samples included in the determination of the sensitivity and specificity
 - Describe sample collection process (include information about timing of sample collection relative to tuberculin injection, when applicable).

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- Describe information to be collected for each animal enrolled in the study and the process for data collection and management.
- Describe how the “true” brucellosis or bovine tuberculosis infection status of animals included in the study will be determined.
- Describe how the proposed test will be evaluated relative to current testing methodologies.
- Describe proposed responsibilities of test manufacturer and VS during the study.
- Owners should be informed about the field study requirements and consent to participate in writing before any animals are tested.
- This written consent should document that the owner understands that:
 - Participation in the field study is voluntary.
 - A new test is being evaluated and the official tests for brucellosis or bovine tuberculosis will be administered at the same time as the test under evaluation.
 - VS may pay indemnity for eligible animals with non-negative (positive or inconclusive) test results.
 - VS will not compensate the owner for participating in the field study.
 - The owner agrees to submit any animals with a non-negative result to the test under evaluation for further testing, even in cases where the official brucellosis or bovine tuberculosis test is negative. The owner agrees to notify VS if an animal with a non-negative test result dies so that specimens can be collected and submitted.
 - Animals that are determined to be non-negative (brucellosis) or suspects (bovine tuberculosis) to official tests will be managed according to brucellosis or bovine tuberculosis program requirements, regardless of negative results on the test under evaluation.
 - If brucellosis or bovine tuberculosis infection is confirmed, the herd will be considered affected and managed according to brucellosis or bovine tuberculosis program requirements.
- CHG staff, in collaboration with CVB, VS District Offices, NVSL, CEAH, and ARS, will analyze the data and evaluate the test's suitability for official program use. CHG staff will summarize the raw data and give an analysis to its collaborators and the manufacturer.
- CHG staff will determine whether the test under evaluation is approved for official program use.
- CVB will inform the company regarding licensure and CHG staff will inform the company whether the test was approved or not approved for official program use. If not approved, CHG staff will offer possible alternatives and next steps.
- CHG staff will prepare official notification, as required by program regulations, to list the test kit as an official test approved for use in the brucellosis or bovine tuberculosis eradication program.

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8.1.1.3 Animal disease classification and indemnity for animals testing non-negative to a test under field evaluation

- The epidemiologist designated by the District Director involved in the field study will report all non-negative test responses to the appropriate CHG specialist and CHG itself.
- If an animal is classified as negative by an official test, but is non-negative on the test under evaluation, the epidemiologist designated by the District Director may classify the animal as a reactor (brucellosis) or suspect (bovine tuberculosis) for purposes of indemnification.
- With the approval of the CHG specialist and conditioned on the availability of funds, VS may provide Federal indemnity for animals classified as reactors or suspects on a test under evaluation and euthanized and necropsied to determine the animal's brucellosis or bovine tuberculosis status and to further validate the test.
 - The epidemiologist designated by the District Director will ensure that the appropriate clinical specimens are collected and submitted. See **Error! Reference source not found.** The epidemiologist designated by the District Director will ensure that large-volume blood samples are collected and submitted to NVSL for banking purposes.
 - The manufacturer may also indemnify non-negative animals and the epidemiologist designated by the District Director will ensure that clinical specimens are collected and submitted.

If not euthanized and necropsied, animals testing non-negative to the test under evaluation may be subject to repeat testing using official brucellosis or bovine tuberculosis tests and the test under evaluation, as determined by the epidemiologist designated by the District Director, CHG, and CHG specialists. Results of this additional testing will determine if herd movement restrictions are necessary. The owner must also agree to notify the designated epidemiologist if the animal is sent to slaughter or dies so that specimens are collected and submitted.

8.1.2 Authorization of State or Federal laboratories to perform brucellosis or tuberculosis diagnostic testing

This section establishes the policy and procedures for approval of laboratories to conduct diagnostic testing for brucellosis caused by *Brucella abortus* and for bovine tuberculosis caused by *Mycobacterium bovis*. It also describes the procedures for maintaining or removing authorization of laboratories, the minimum requirements for laboratories requesting approval, and the procedures for APHIS District offices to follow when responding to such requests.

Both VS and States or Tribes approve and authorize Federal, State, or university laboratories to perform brucellosis and bovine tuberculosis official diagnostic testing. (Laboratories approved by the National Animal Health Laboratory Network may also be authorized as official testing laboratories.) These laboratories are granted authorization based on a programmatic need to have additional testing capacity and on having adequate facilities, testing protocols, and competent personnel. Proficiency testing, laboratory inspections, and/or paper audits may be required.

8.1.2.1 Initial laboratory approval

- Request for approval: Laboratories should make all requests for initial approval to the appropriate AD for their State or Tribe. The AD will consult with the SAHO or Tribal animal health official regarding the request.
- Submission package: Laboratories requesting approval should submit an original and three copies of their request to the District Office for their State or Tribe. The submission package consists of the laboratory's responses to the following items:
 - Name and address of the laboratory
 - Name of the legally responsible official and, if different, the name of the laboratory director
 - A description of the facilities and equipment the laboratory will use to perform the brucellosis or bovine tuberculosis official diagnostic tests. The equipment must be generally accepted as appropriate for the test(s) that will be conducted
 - A list of the types of diagnostic samples that will be tested (blood, milk, etc.)
 - A list of the specific brucellosis or bovine tuberculosis official diagnostic tests for which the laboratory requests approval
 - A detailed description of the brucellosis or bovine tuberculosis official diagnostic test procedures
 - A list of the individuals performing brucellosis or bovine tuberculosis official diagnostic tests and their qualifications. Every laboratory should have a primary and a backup operator for conducting the specific tests for which they are requesting approval. Individuals conducting these tests must be tested annually to determine their competence to perform the approved procedures. The minimum requirements for those individuals are:
 - Knowledge, skills, and abilities related to the laboratory procedures required for the test
 - Proficiency and accuracy in testing a panel of appropriate samples from animals of known disease status. Personnel will demonstrate their proficiency by testing a panel of appropriate samples as required by NVSL. The NVSL Director, will provide the test panels and determine the appropriate schedule for proficiency testing. User fees for the test panels may be applicable.
 - The individual(s) providing laboratory oversight of brucellosis or bovine tuberculosis specific official diagnostic testing must have a degree in immunology, microbiology, or veterinary medicine, or must demonstrate that they have sufficient experience in those fields or a related field to provide the necessary background.
- Minimum requirements for approval to conduct brucellosis or bovine tuberculosis serology:
 - Demonstrated knowledge, skills, and abilities in the laboratory procedures required.
 - Proficiency and accuracy in testing a panel of appropriate samples from animals of known disease status. (The maintenance of this proficiency and accuracy will be

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- demonstrated by testing similar panels. Schedule of proficiency testing to be determined by NVSL.).
- For laboratory oversight of serological testing or for interpreting results, individuals must also have a degree in immunology, microbiology, veterinary medicine, or must demonstrate that they have sufficient experience in those fields or a related field to provide the necessary background.
 - Documentation of any brucellosis and bovine tuberculosis serology proficiency testing programs available from NVSL. *Note:* Participation in the laboratory proficiency test program conducted by NVSL is not a requirement for submitting the *initial* package. However, all individuals at the laboratory that will be performing the test will participate in the appropriate NVSL proficiency testing program before it is approved. Each individual in the laboratory must pass an approved proficiency test prior to conducting official tests, including new employees that are hired after the laboratory is approved. (Please note: Under certain situations, there will be a NVSL user fee associated with proficiency testing kits.)
 - A description of the specific procedures, including timeframe, for reporting test results.
 - Upon receiving a request for laboratory approval, the AD will inform the SAHO or Tribal animal health official that the request has been submitted. The SAHO or the Tribal animal health official and the AD may determine that an additional laboratory is not needed and may reject the request. If they decide to submit the package, they will inform the District Director of the request. The District Director will inform CHG staff and the NVSL Director. This group will decide by consensus if a need exists for an additional approved laboratory. VS will consider the ability to support an additional laboratory without negative resource impact to NVSL in making its decision.
 - If they determine that a need exists, the AD must inform the requesting laboratory of the requirements for approval by providing a copy of this document. The AD must discuss the requirements for laboratory approval with the director of the laboratory or other appropriate individual. The AD may request the director of the laboratory consult with NVSL as well.
 - Upon receiving the laboratory's submission package, the AD must review the package to determine if it is complete and satisfactory. The AD should review the package while consulting with the SAHO or Tribal animal health official. If there are deficiencies, the AD will return the submission package to the laboratory, clearly stating the reasons for VS' denial. The AD will invite the laboratory to remedy the deficiencies and resubmit the package.
 - After receiving a satisfactory submission package, the AD or his or her representative must conduct a site visit. If requested, NVSL will assist with the inspection. During the site visit, the AD must again review the requirements for laboratory approval, review the test procedures the laboratory intends to use, and confirm that the information in the

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submission package is correct. The Biosafety in Microbiological and Biomedical Laboratories, 5th edition should be used to evaluate both procedures and facilities.

8.1.2.3 Recommendation for approval

- After reviewing the laboratory's submission package and conducting the site visit, the AD, with the concurrence of the SAHO or the Tribal animal health official, may recommend approval of the laboratory. The original submission package and the AD's letter of recommendation are sent to the NVSL Director, with copies to the District Director and the brucellosis or bovine tuberculosis program staff.
- The NVSL Director will review the submission package for compliance with established scientific guidelines.
 - If the submission package is satisfactory, the NVSL Director will notify the District Director, the AD, the laboratory, and CHG staff that the laboratory will be approved pending completion of laboratory proficiency testing for the specific tests listed in the package.
 - If the submission package is unsatisfactory, the NVSL Director will indicate the deficiencies in writing and send this information to the District Director, the AD, the director of the laboratory requesting approval, and CHG staff.
 - If the laboratory wishes to reapply for approval, it must submit a corrected package in quadruplicate to the AD, who will then forward copies to the District Director, the NVSL Director, CHG staff.
- Laboratory Proficiency Testing: As noted above, prior to being approved by the NVSL Director, laboratories requesting to conduct specific brucellosis serologic tests, or TB specific serologic tests will be required to subscribe to and pass a proficiency testing program approved by NVSL.

8.1.2.4 Changes to laboratory approval

- A laboratory must notify the AD of any changes in the submission package information, excluding new personnel. Laboratories should submit amendments to the package in quadruplicate to the AD. The AD will review the amendments and may recommend approval or may elect to conduct a new site visit. If the AD recommends approval of the proposed changes, copies of the proposal must be sent, along with a recommendation letter, to the District Director, the NVSL Director, and CHG staff.
- If the AD cannot recommend approval of the proposed changes, he or she must send a letter stating the reasons for disapproval to the requesting laboratory, the District Director, the NVSL Director, and CHG staff.
- The NVSL Director must decide whether to approve the amended package and then must notify the AD, the requesting laboratory, the District Director, and CHG staff.

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- If the NVSL Director cannot recommend approval of the proposed changes, he or she must send a letter stating the reasons for disapproval to the requesting laboratory, the District Director, the AD, and CHG staff.

8.1.2.5 Maintaining laboratory approval

To maintain approval, the laboratory must demonstrate continual adherence to the standards under which it was approved. To that end, personnel performing the tests are required to participate in NVSL's proficiency testing program and satisfactorily complete proficiency tests. Persons not achieving satisfactory results on a proficiency test must suspend testing until there have been documented re-training efforts and ability to pass a proficiency check test. NVSL will notify the AD, the District Director, and CHG staff whether or not the laboratory meets the proficiency requirements.

Additionally, VS may conduct a review of the laboratory's conditions or procedures at any time to determine such adherence.

8.1.2.6 Revocation of laboratory approval

- VS may revoke laboratory approval upon the approved laboratory's request.
- Unsatisfactory performance on required proficiency tests may cause VS to revoke approval.
- A finding of unsatisfactory conditions or procedures at the laboratory may cause VS to revoke approval.
- A determination that the laboratory substantially falsified information on its application for approval may cause VS to revoke approval.
- When any of the criteria for approval or maintaining approval are no longer met, VS may withdraw approval on recommendation of the AD, the District Director, the NVSL Director, or CHG staff.
- If VS approves the laboratory to perform more than one diagnostic test procedure, VS may revoke approval either for a specific test procedure, for all approved procedures, or for a particular individual. The laboratory will be promptly informed of the revocation and reasons for this action.
- The NVSL Director, acting on behalf of the Administrator, will provide written notice of a proposed revocation of approval, including the reasons for the proposed revocation, to the laboratory director. This notice will include information on how the laboratory can provide a response. Any conflicts concerning the reasons for revoking the approval will be resolved by a meeting of the NVSL Director, the requesting laboratory's Director, CHG's Director, and their respective subject matter experts.

8.1.2.7 List of approved laboratories

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- The NVSL Director will maintain a list of laboratories approved to conduct official brucellosis or bovine tuberculosis diagnostic tests. The approved list of laboratories approved to conduct official testing is posted to at: www.aphis.usda.gov/nvsl under the approved labs link.
- The NVSL will notify approved laboratories when the standard operating procedure (SOP) for that test is updated.
- Standard operating procedures for all official tests can be obtained from NVSL by contacting at 515-337-7568 or nvsl.mastercontrol@aphis.usda.gov

8.2 Diagnostic tests and interpretation for bovine brucellosis

Table 1 table lists the various official tests, supplemental tests, and other types of diagnostic tests used for bovine brucellosis.

Table 2 Brucellosis tests

• Stage	• Brucellosis Tests
• Screening	<ul style="list-style-type: none"> • Rapid Automated Presumptive (RAP) • Buffered Acidified Plate Antigen (BAPA) • Card test – for First Point Testing and/or a specified field use, if approved by the Assistant Director (AD). • Fluorescent Polarization Assay (FPA) • Brucellosis Ring Test (BRT) • Heat Inactivation Ring Test (HIRT)
• Secondary	<ul style="list-style-type: none"> • Fluorescent Polarization Assay (FPA) • Complement Fixation (CF) • Organism Detection tests such as culture isolation and identification.
• Supplemental	<ul style="list-style-type: none"> • Standard Plate Test (SPT) • Standard Tube Test (STT) • Enzyme-Linked Immunosorbent Assay (ELISA) • Western Blot Test • Rivanol • Genotyping
• Postmortem	<ul style="list-style-type: none"> • Post mortem examination/necropsy, including the collection of tissues and samples for culture and identification

8.2.1 Use of tests and test interpretation tables for brucellosis

Test interpretation tables are generally accepted guidelines to be used to interpret test results. These test results, along with other appropriate epidemiological investigation information, are

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used by the epidemiologist designated by the District Director to classify the animal's brucellosis status. All First Point Testing (FPT) and field screening test results must be confirmed at a cooperative State/Tribal-Federal approved brucellosis laboratory.

8.2.1.1 Classification of cattle, bison, and captive cervids – Serologic tests

- Animals are classified as “negative,” “suspect,” or “reactor” based in part on their serologic test results, using the criteria in the test interpretation tables for classification.
- Titer responses for all cattle, bison, and captive cervids must be evaluated by a trained, experienced epidemiologist, who has been designated to perform and/or supervise this function in each of the States.
- The brucellosis epidemiologist designated by the District Director must take into consideration the animal and herd history as well as other epidemiologic factors.
- The brucellosis epidemiologist designated by the District Director should take into account factors, including but not limited to, epidemiological investigation information, vaccination status, and specific State of origin and State of destination regulations.
- The brucellosis epidemiologist designated by the District Director has the authority to deviate from the suspect or reactor criteria but must document the reasons for doing so and should consult with the CHG specialist before doing so.

8.2.2 Brucellosis screening tests

8.2.2.1 Rapid automated presumptive test (RAP)

- RAP is an automated serologic test to detect the presence of *Brucella* antibodies in test-eligible cattle, bison, and captive cervids.
- RAP utilizes a scanning auto-reader that measures alterations in light transmission through each test well and the degree of agglutination present.
- RAP test results are interpreted as either negative or positive.
- Cattle, bison, and cervids negative to the RAP test are classified as brucellosis negative.
- Cattle, bison, and cervids positive to the RAP test shall be subjected to official secondary tests to determine their brucellosis infection classification.
- For additional information, see the Serologic Assays Manual.

8.2.2.2 Buffered acidified plate antigen (BAPA)

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- BAPA is an approved screening test for any bovine, bison or captive cervid serum samples submitted to a cooperative State/Tribal-Federal brucellosis laboratory (except sera to be tested for international export purposes) to determine the brucellosis infection status of test-eligible cattle, bison, and captive cervids.
- BAPA test results are interpreted as either negative or positive.
- Cattle, bison, and captive cervids negative to the BAPA test are classified as brucellosis negative.
- Cattle, bison, and captive cervids positive to the BAPA test shall be subjected to official secondary tests to determine their brucellosis infection classification.
- Use of the BAPA at livestock markets: The BAPA test can be used to classify cattle negative at State/Tribal-Federal laboratories or at livestock markets. All samples negative to the BAPA at livestock markets shall be retested at an approved brucellosis laboratory for verification. All samples positive to the BAPA at the livestock market shall be retested with an official secondary test at an approved brucellosis laboratory.
 - In livestock markets, the official testing of cattle samples classified as positive to the BAPA test may be accomplished by one of the following options:
 - Option A: Hold the affected animals until results from a cooperative State/Tribal-Federal brucellosis laboratory are reported; or
 - Option B: Retest the serum with the secondary FPA test.
 - The status of the positive animal and the other animals in the same herd or lot is determined by the results obtained at the livestock market (option B) or at the State/Tribal-Federal brucellosis laboratory using official tests (option A).
 - The Assistant Director will immediately review the laboratory procedures with cooperating State or Tribal officials in preparation for initiating this policy; i.e., (1) the use of the brucellosis card test as a screening test in cooperative State-Federal laboratories is terminated in favor of the BAPA test or RAP; and (2) provide suitable training and equipment for the BAPA test to the livestock market veterinarian so that the brucellosis card test in livestock markets is minimized by using the BAPA test as a preliminary screening test.
- For additional information, see the Serologic Assays Manual

8.2.2.2 *Buffered acidified plate antigen (BAPA)*

- BAPA is an approved screening test for any bovine, bison or captive cervid serum samples submitted to a cooperative State/Tribal-Federal brucellosis laboratory (except sera to be tested for international export purposes) to determine the brucellosis infection status of test-eligible cattle, bison, and captive cervids.
- BAPA test results are interpreted as either negative or positive.

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- Cattle, bison, and captive cervids negative to the BAPA test are classified as brucellosis negative.
- Cattle, bison, and captive cervids positive to the BAPA test shall be subjected to official secondary tests to determine their brucellosis infection classification.
- Use of the BAPA at livestock markets: The BAPA test can be used to classify cattle negative at State/Tribal-Federal laboratories or at livestock markets. All samples negative to the BAPA at livestock markets shall be retested at an approved brucellosis laboratory for verification. All samples positive to the BAPA at the livestock market shall be retested with an official secondary test at an approved brucellosis laboratory.
 - In livestock markets, the official testing of cattle samples classified as positive to the BAPA test may be accomplished by one of the following options:
 - Option A: Hold the affected animals until results from a cooperative State/Tribal-Federal brucellosis laboratory are reported; or
 - Option B: Retest the serum with the secondary FPA test.
 - The status of the positive animal and the other animals in the same herd or lot is determined by the results obtained at the livestock market (option B) or at the State/Tribal-Federal brucellosis laboratory using official tests (option A).
 - The Assistant Director will immediately review the laboratory procedures with cooperating State or Tribal officials in preparation for initiating this policy; i.e., (1) the use of the brucellosis card test as a screening test in cooperative State-Federal laboratories is terminated in favor of the BAPA test or RAP; and (2) provide suitable training and equipment for the BAPA test to the livestock market veterinarian so that the brucellosis card test in livestock markets is minimized by using the BAPA test as a preliminary screening test.

For additional information, see the Serologic Assays Manual

8.2.2.3 Card test

- An official brucellosis program test that can be used to determine the brucellosis infection status of cattle, bison and captive cervids for interstate movement of cattle, bison and captive cervids from a specifically approved livestock facility in a State where first point testing (FPT) is conducted and at VS approved brucellosis diagnostic laboratories. See [9 CFR 71.20](#) for requirements to be an approved livestock facility. It may also be used in the field for a specified field use, if approved by the AD.
- The card test may also be used as a supplemental test conducted in an approved laboratory to give the epidemiologist designated by the District Director additional information when classifying cattle, bison, and captive cervids.
- Standard card test results are interpreted as either negative or positive.
 - A moderate to marked clumping agglutination reaction is a positive result.

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- Test-eligible cattle, bison and captive cervids positive to the standard card test and shall be subjected to official brucellosis secondary testing to determine their brucellosis infection classification.
- Test-eligible cattle, bison and captive cervids negative to the standard card test are classified as brucellosis negative.

8.2.2.4 *Fluorescent Polarization Assay (FPA)*

- An official brucellosis program test that can be used to determine the brucellosis infection status of cattle, bison and captive cervids for interstate movement of cattle, bison and captive cervids from a specifically approved livestock facility in a State where first point testing (FPT) is conducted and at VS approved brucellosis diagnostic laboratories. See [9 CFR 71.20](#) for requirements to be an approved livestock facility. It may also be used in the field for a specified field use, if approved by the AD. See 8.3.2.1 for additional information regarding the FPA test.

.2.3 Brucellosis secondary tests

8.2.3.1 *Fluorescent Polarization Assay (FPA)*

- FPA is a quantitative serologic test to determine the brucellosis status of test-eligible cattle, bison, and captive cervids when conducted according to instructions approved by APHIS.
- FP assays are interpreted as either positive, negative, or suspect.
 - Cattle, bison, and captive cervids negative to the FP assay are classified as brucellosis negative.
 - Cattle, bison, and captive cervids with positive FP assay results are classified as brucellosis reactors, while cattle, bison, and captive cervids with suspect FP assay results are classified as brucellosis suspects.

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Table 3 Interpretation of FPA testing

Cattle, Bison, and Cervids

(10 and 20 microliter samples) mP = millipolarization units, Delta mP = sample mP – mean negative control mP

<u>Delta mP result recorded on forms</u>	<u>Test Interpretation</u>
Delta mP value	Negative (delta mP 1-10)
Delta mP value	Suspect (delta mP 11 – 20)
Delta mP value	Suspect (delta mP 11 – 40) Provided the CF test results are negative
Delta mP value	Positive (delta mP \geq 21) Provided the CF test results are positive, anti-complementary, or not available)
Delta mP value	Positive (delta mP \geq 41) Regardless of CF test results

Note: FPA test results will be recorded as the delta mP titer (titer minus the negative control titer) of the specimen. For example, if a specimen had a titer of 125 and the negative control titer was 85, then the delta mP result will be recorded on the VS 4-54 or VS 4-33 as “40”.

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8.2.3.2 Complement-Fixation (CF) test

- CF is a test to determine the brucellosis infection status of test-eligible cattle, bison and captive cervids when conducted according to instructions approved by APHIS and the State or Tribe in which the test is to be conducted.

Table 4 Interpretation of manual CF test results

Compliment Fixation Test (CF)	
Test Result Recorded on Forms	Test Interpretation (Do Not Record on Forms)
1+ 1:10 or lower	Negative
2+ 1:10 through 1+ 1:20	Suspect
2+ 1:20 or higher	Reactor
AC	Indeterminate

Key to degree of hemolysis: 1+ = 75 percent, 2+ = 50 percent, 3+ = 25 percent, 4+ = 0 percent.

Note: CF dilution results shall be reported as follows, e.g. 1+ 1:10, 2+ 1:20, etc. shall be recorded on the VS 4-54 or VS 4-33 or VS 10-4 (NVSL).

AC = anti-complementary result = indeterminate result. The factors causing an anti-complementary result are not known but the test may be successful with a new sample from the animal. Please submit clear serum, without evidence of hemolysis, for complement fixation or other serologic tests.

8.2.4 Brucellosis supplemental tests

8.2.4.1 Standard plate test (SPT) or standard tube test (STT)

- SPT or STT is as a supplemental test in an approved laboratory to give the epidemiologist designated by the District Director additional information when classifying cattle, bison, and captive cervids when conducted according to instructions approved by APHIS and the State or Tribe in which the test is to be conducted.
- Cattle, bison and captive cervids are classified according to the following agglutination reactions:

Table 5 Interpretation of STT and SPT results

Test Results			Test Interpretation
1:50	1:100	1:200	
–	–	–	Negative
I	–	–	Suspect
+	–	–	Suspect
+	I	–	Suspect
+	+	–	Reactor
+	+	I	Reactor
+	+	+	Reactor

Key: Negative (-) = no agglutination; Incomplete (I) = incomplete agglutination; Positive (+) = complete agglutination

8.2.4.2 Enzyme-Linked Immunosorbent Assay (ELISA)

- This test may be performed by the NVSL for export testing when requested by the submitter.
- The test uses either serum or milk samples.

8.2.4.3 Western blot test

- The Western blot test, also called immunoblotting, is a test for a specific protein within a protein mixture. The Western blot test is performed after gel-electrophoresis or an enzyme-linked immunosorbent assay (ELISA) test, and it uses antibodies to identify specific proteins.
- The test can be used a supplemental test to provide additional analysis of the results for epidemiological investigations.

8.2.4.4 Rivanol test

- The Rivanol test is used to as a supplemental test in an approved laboratory to give the epidemiologist designated by the District Director additional information when classifying cattle, bison, and captive cervids when conducted according to instructions approved by APHIS and the State or Tribe in which the test is to be conducted.
- Cattle, bison and captive cervids are classified according to the following agglutination reactions:

Table 6 Interpretation of Rivanol test results

Test Results	Test Interpretation
1:25 or lower	Negative
Not Applicable	Suspect
+ 1:25 or higher	Reactor

8.2.4.5 Genotyping

- Genotyping using whole genome sequencing is a high resolution method for determining the relatedness of isolates.
- Laboratories recovering isolates suspicious of or identified as *Brucella* sp. from livestock species should submit them to NVSL for genotyping and archiving according to current SOPs.

8.2.5 Brucellosis milk surveillance tests

8.2.5.1 Standard brucellosis ring test (BRT)

- The standard BRT is performed on pooled milk from dairy herds and represents all of the lactating cows that contributed milk to that sample.
- In States or Tribes in which BRT testing is not required, there is a user fee associated with this test and the reagents necessary to run the test.
- Collection procedures— If States or Tribes require BRT testing, samples of pooled milk from pipeline segments, bulk tanks, or milk cans are to be collected at a frequency determined by the State or Tribe at milk receiving stations, dairy processing plants, or individual dairy herds in each State or Tribe.
 - Records should be kept for each collection point on each collection indicating how the samples were handled by the dairy plant after the samples were collected at the farm and before subsamples were collected for the BRT.

8.2.5.2 Serially diluted BRT

- The serially diluted BRT procedure is used by brucellosis epidemiologists designated by the District Director to evaluate the level of antibodies in the milk of individual cows. This test is commonly performed on separate milk samples from individual quarters.
- This special test is often combined with bacteriologic attempts to culture *Brucella* from aseptically collected milk samples.

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8.2.5.3 *The heat-inactivated ring test (HIRT)*

- This test is an approved test for milk and/or cream samples whose results from the BRT are suspicious.
- The interpretation of HIRT results must be made in conjunction with other epidemiologic factors by a brucellosis epidemiologist designated by the District Director.

8.2.5.4 *The California BRT*

- The California BRT was developed to reduce the resources necessary when segmenting large dairy herds. It is performed on pooled milk from dairy herds and represents all of the lactating cows that contributed milk to that sample.
- Collection and test procedures are the same as the standard BRT with the exception of the amount of milk, and the schedule of testing, which is at a frequency determined by the State or Tribe.

8.2.6 Disposition of outdated *Brucella* reagents

- *Brucella* reagents (antigens and control sera) must not be used beyond the assigned expiration date; however, expiration date extensions may be granted to reagent users by the NVSL *Brucella* and *Mycobacterium* Reagents Team. Written notification from NVSL regarding extensions will be sent by fax and/or other electronic communication to reagent users.
- To avoid overstock situations, limit the order amounts of reagents to no more than a 3-month supply. Reagents with the shortest expiration dates should be used first.
- Contact the NVSL *Brucella* and *Mycobacterium* Reagents Team at (515) 663-7181 if a reagent supply will not be used before the expiration date. The Reagents Team will either request users to return the reagent to NVSL, request users to send the reagent to an alternate source for use before the expiration date, extend the expiration date, or request disposal by the user.
- If NVSL requests disposal of the reagent by the user, each reagent's data sheet should be referenced for product details. Additionally, user disposal of each product should be handled in accordance with the facility's chemical and waste disposal policies.

8.2.7 Use of the brucellosis card test

- APHIS will fund the use of the card test for first point testing in States or Tribes that have a recognized management area.

Any State or Tribe conducting FPT must have a memorandum of understanding (MOU) with VS regarding the use of the brucellosis standard card test.

In States or Tribes where VS is funding FPT, federally accredited veterinarians authorized to perform FPT at specifically approved stockyards and authorized State/Tribal and Federal brucellosis program personnel may obtain brucellosis standard card test kits and antigen from NVSL at no charge.

- All orders for brucellosis standard card test kits, cards, and antigen must be submitted to NVSL.
- In States or Tribes where VS is not funding FPT, federally accredited veterinarians authorized to perform FPT at specifically approved stockyards and authorized State/Tribal and Federal brucellosis program personnel will be charged a user fee and must establish a user fee account with NVSL to obtain the brucellosis standard card test kits and antigen.
 - All orders for brucellosis standard card test kits, cards, and antigen must be submitted to NVSL.
- All blood samples collected to perform the brucellosis standard card test at a VS specifically approved stockyard or in the field must be retested at a VS-approved brucellosis diagnostic laboratory. ²
- If APHIS is not funding FPT in a State or Tribe, the shipment of these samples will be at State or Tribe expense. All States or Tribes conducting FPT will report all brucellosis standard card test results, positive or negative, within a timeframe specified in the MOU to the appropriate AD.
- An APHIS-approved brucellosis diagnostic laboratory may charge a fee for brucellosis testing of blood samples received from other States or Tribes where APHIS is not providing funding for FPT.

The brucellosis standard card test may only be used in a State or Tribe having a signed MOU with VS by federally accredited veterinarians and State/Tribal and Federal brucellosis program personnel who have been trained, qualified, and authorized.

² The current VS-approved brucellosis diagnostic laboratories list is available at http://www.aphis.usda.gov/animal_health/lab_info_services/approved_labs.shtml.

8.2.7.1 Policy on use of the brucellosis standard card test

- The brucellosis standard card test, using buffered *Brucella* antigen, is to be used as the official brucellosis test for cattle or bison for FPT, if the SAHO or Tribal animal health official has specifically designated this test as the official test for cattle and bison tested at all VS specifically approved stockyards in that State or Tribe.
- When conducted at VS specifically approved stockyards, the brucellosis standard card test is recognized as the official test for the purposes of interstate movement.
- The SAHO or Tribal animal health official and AD will issue and sign an MOU outlining the purposes for which the brucellosis standard card test can be used including use by State and Federal brucellosis program personnel other than at VS specifically approved stockyards.

8.2.7.2 Memorandum of understanding for use of brucellosis standard card test

The brucellosis standard card test may be used only within a State under an MOU signed by the AD and the SAHO and approved by the VS District Director.

Appendix 12: Memorandum of Understanding

- Policy statement regarding the use of the brucellosis standard card test in official brucellosis program work:
 - Purpose for using brucellosis standard card test; e.g., designating it as the official test at all VS specifically approved stockyards for the purpose of interstate movement, a screening or supplemental test in a VS-approved brucellosis diagnostic laboratory on bovine and bison sera, and other specified use by State and Federal brucellosis program personnel.
 - Location and conditions for using the brucellosis standard card test; i.e., VS-approved brucellosis diagnostic laboratories and FPT.
 - Terms for testing cattle and bison: Use of the brucellosis standard card test is restricted because it can be misused as an illegal screening test. The brucellosis standard card test is generally restricted for use in VS-approved brucellosis diagnostic laboratories. However, State/Tribal and Federal brucellosis personnel may be authorized to use the brucellosis standard card test under other circumstances that the AD has approved. In States or Tribes where the brucellosis standard card test has been designated as the official test at all VS specifically approved stockyards, authorized federally accredited veterinarians may only use the brucellosis standard card test in VS specifically approved stockyards to determine the brucellosis infection status of cattle and bison for interstate movement.
- Personnel authorized to conduct the brucellosis standard card test:

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- Only authorized State/Tribal and Federal brucellosis program personnel may conduct the brucellosis standard card test on cattle and bison at premises other than VS-approved livestock facilities. Authorized federally accredited veterinarians may use the brucellosis standard card test for testing sale cattle and bison at VS specifically approved stockyards if the signed, approved MOU designates the brucellosis standard card test as the official test for cattle and bison at all VS specifically approved stockyards in the State or Tribe.
- The SAHO or Tribal animal health official and AD will designate two qualified persons as "training officers" within each State and Tribe to train personnel in conducting the brucellosis standard card test and performing annual proficiency tests. The SAHO or Tribal animal health official and AD may designate additional training officers, if needed to meet program needs and if approved by the Deputy Administrator.
 - The designated training officers will continually review initial and confirmation tests performed at VS-approved brucellosis diagnostic laboratories to determine if the authorized persons retain proficiency to accurately diagnose both positive and negative cattle and bison sera.
- Federally accredited veterinarians and State/Tribal and Federal animal health personnel authorized to conduct brucellosis standard card testing of cattle and bison sera will take an annual proficiency test.
- Annually, before renewal of the authorization, the authorized persons will be required to satisfactorily conduct and interpret the brucellosis standard card test on standardized proficiency test sera using personal equipment (e.g., brucellosis standard card test rocker and cover) at either a designated laboratory or at the field site (VS specifically approved stockyards, etc.).
- The AD will maintain a signed copy of all brucellosis standard card test notices, brucellosis standard card test authorizations, and MOUs regarding the brucellosis standard card test.
 - A signed brucellosis standard card test notice is required each time a brucellosis standard card test kit or antigen is shipped to an authorized individual.
 - The AD will give the training officers a list of authorized State/Tribal and Federal brucellosis program personnel and federally accredited veterinarians that includes the anniversary date of their authorization.

8.2.7.3 Brucellosis standard card test authorizations

- A federally accredited veterinarian may submit a written request for brucellosis standard card test authorization to the AD. The AD, in consultation with the SAHO or Tribal animal health official, will approve or disapprove the request.
- Authorization process:
 - The AD and SAHO or Tribal animal health official will jointly agree upon and designate training officers to train brucellosis program personnel and federally accredited veterinarians in performing and reading the test to maintain uniformity.

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- The training officer will submit a record of persons who have been trained and qualified to conduct the test to the AD.
- The AD will establish an annual authorization system for authorizing federally accredited veterinarians and State/Tribal and Federal program personnel to conduct the brucellosis standard card test and will monitor authorized use of the brucellosis standard card test.
- The AD issues the authorization to federally accredited veterinarians and State/Tribal and Federal personnel to receive and use the brucellosis standard card test kit, antigen, and any restricted supplies and equipment either directly or through the office of the SAHO or Tribal animal health official.
- Authorized brucellosis card test users are required to complete and sign a brucellosis standard card test notice acknowledging receipt of brucellosis standard card test kits and antigens issued and maintain such kits and antigen to prevent any unauthorized use.
- Conditions for authorization renewal:
 - Successful completion of annual proficiency tests.
 - Consistent submission of quality blood samples for confirmatory testing.
 - Submission of proper test records containing complete individual animal identification for test confirmation at a designated laboratory.
 - Continued use of the brucellosis standard card test in accordance with the conditions for authorized use.
- Suspension and Revocation of Authorization: The brucellosis standard card test kit and antigen are the exclusive property of the U.S. Government. To ensure the integrity and continued effectiveness of the Federal-State brucellosis eradication program, the AD may initiate an APHIS Investigative and Enforcement Services (IES) investigation of an authorized user if it is suspected the conditions for use of the brucellosis standard card test have not been met or the test, as described in the MOU, is being used improperly. After receiving the IES investigation report:
 - If violations of the conditions of use are reported, the AD will inform the authorized users in writing of specific instances that may fail to meet the conditions of authorized use and that may result in suspension or revocation of their accreditation or authorized use.
 - The written notice will give the authorized user 20 days to respond in writing to the allegations and to request an informal conference with the SAHO or Tribal animal health official and the AD. The AD will arrange the time and place of the conference.
 - If the authorized user does not respond to the written notice and does not request an informal conference within 20 days, the AD will issue an accreditation revocation or authorized use revocation order. The authorized use will be revoked for at least 12 months. The person may reapply for a brucellosis standard card test authorization after the revocation period.
 - The AD will revoke authorized use if the authorized user consents in writing to the order revoking or suspending authorized use before or during the informal conference. Alternatively, the AD may issue a letter of warning to the authorized user if that is determined to be sufficient to obtain compliance after the informal

- conference. If the parties are unable to agree to the issuance of a letter of warning or a consent order, the AD will submit the proceedings of the informal conference and investigation report to the Deputy Administrator for resolution. When necessary to protect the public health, interest, or safety, the Deputy Administrator, upon the recommendation of the AD, may make a summary suspension effective immediately upon written notification. A summary suspension made effective immediately upon written notification will remain in effect until official resolution has been made.
- A federally accredited veterinarian's authorization to use the brucellosis standard card test and antigen will be automatically suspended or revoked if the veterinarian's Federal accreditation is suspended or revoked.

8.2.7.4 Distribution of brucellosis standard card test kits and antigen

- VS will issue brucellosis standard card test kits and antigen for brucellosis testing of cattle and bison sera to authorized federally accredited veterinarians for performing authorized tests only at VS specifically approved stockyards and to authorized State and Federal animal health employees.
- Authorized federally accredited veterinarians and authorized State/Tribal and Federal brucellosis program personnel must complete a brucellosis standard card test notice.
- Before purchase and shipment, the AD will review and approve the completed brucellosis standard card test notice and fax it to NVSL to verify that the order is approved. The AD will also indicate on the brucellosis standard card test notice to whom the order is to be charged and provide the shipping address.
- The AD will give NVSL a list of authorized users.
- Inventory control:
 - The SAHO or Tribal animal health official and the AD will determine and agree upon whether the State/Tribe or AD will maintain inventory control of the brucellosis standard card test kits and antigen in their State or Tribe.
 - At least 60 days before the antigen's expiration date, the entity (AD or State/Tribe) responsible for inventory control will contact NVSL to determine if an extension of the antigen's expiration date has been approved.
 - If an extension of the antigen's expiration date has not been approved, NVSL will direct the responsible entity (State/Tribe or AD) to transfer the antigen to another station for more rapid use or to properly dispose of the antigen.
 - If the State or Tribe is responsible for inventory control, it will report any antigen that expires to the AD.
 - The AD will report any antigen that expires to the Director of the appropriate VS District.
 - Authorized users who are issued brucellosis standard card test kits and antigen must give an accounting to the AD and SAHO or Tribal animal health official for all cards and antigen issued.

- Each State or Tribe must have a system to record the number of blood samples tested by authorized users. The system may require each person to document tests conducted before VS issues additional kits or require the VS-approved brucellosis diagnostic laboratories to maintain records on the number of blood samples submitted.
- VS will not issue additional brucellosis standard card test kits and antigen unless the number of samples submitted for confirmation corresponds to the number of tests available in previously issued kits. The AD should consider initiating an IES investigation if unexplainable discrepancies are revealed.

8.3 Submission of tissue and/or milk samples to NVSL– brucellosis

To confirm diagnosis of brucellosis and establish pathogenicity, VS or State/Tribal employees must submit all samples for isolation, identification, and genotyping to the NVSL. NVSL has established [standard procedures](#) for the submission of such samples. Before these personnel submit samples, the AD must approve the submission.

8.4 Official tests for bovine tuberculosis

The following tests have been granted program approval and are considered official tests for bovine tuberculosis when used in the manner and species specified.

Table 7 Official antemortem tests for bovine tuberculosis

Stage	Official bovine tuberculosis Tests in Cattle and Bison	Official bovine tuberculosis Tests in Captive Cervids
Screening	<ul style="list-style-type: none"> • Caudal fold tuberculin (CFT) test • Cervical test (CT) • IDEXX M. bovis Ab test in TB affected cattle herds only. 	<ul style="list-style-type: none"> • Single cervical tuberculin (SCT) test • Dual Path Platform (DPP) test
Secondary	<ul style="list-style-type: none"> • Comparative cervical tuberculin (CCT) test • Bovine gamma interferon test (CATTLE ONLY) 	<ul style="list-style-type: none"> • Comparative cervical tuberculin (CCT) test • Dual Path Platform (DPP) test

8.4.1.1 Johne’s vaccination

Cattle vaccinated for Johne’s disease, Mycobacterium avium sp. paratuberculosis, may exhibit sensitivity to M. bovis PPD. In most cases, a differential diagnosis can be made by conducting the comparative cervical or gamma interferon test.

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Before vaccinating a herd for Johne’s disease the herd must have a negative *M. bovis* history and shall have a negative TB test negative prior to initiating vaccinations. Purchased replacement stock should have a negative tuberculin test before introduction into the herd.

8.4.1.2 Use of PPD tuberculin in official bovine tuberculosis skin tests

PPD tuberculins are used to conduct the CFT, SCT, CT, and CCT tests, as follows:

Table 8 PPD tuberculins available for official bovine tuberculosis testing

Test	PPD Used	Standardization
<ul style="list-style-type: none">• Caudal fold tuberculin (CFT) test• Single cervical tuberculin (SCT) test	Bovine PPD Tuberculin (PPD Bovine) Available in 1 ml, 5 ml, and 10 ml vials	1.0 mg protein/ml
<ul style="list-style-type: none">• Cervical test (CT)	Bovine Cervical Test PPD Tuberculin (PPD Bovine, Cervical) Available in 2 ml vials.	2.0 mg protein/ml
<ul style="list-style-type: none">• Comparative cervical tuberculin (CCT) test	Balanced PPD Tuberculins (PPD Bovine and PPD Avian)	Matched pairs with protein concentrations adjusted to equalize their biological activity.

8.4.1.3 Obtaining PPD tuberculin from the USDA

Reagent requests should be submitted utilizing VS Form 4-9. This form can be found [here](#).

8.4.1.4 PPD tuberculin storage and handling

- Official testers must properly store and handle PPD tuberculin to maintain its potency and ensure consistently accurate testing. The following procedures are recommended:
 - Tuberculins are to be stored at temperatures between 35 and 45 degrees Fahrenheit (2 to 7 degrees Celsius). Do not allow tuberculins to freeze, as the tuberculous protein will precipitate and may cause variations in the response. Tuberculin that has been frozen should not be used and should be properly discarded, as set forth in VS Memorandum 800.56, “Disposal of Unsatisfactory and Undesirable Materials.” Tuberculins are fairly stable at room temperature.

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- Tuberculin is to be stored in a dark location. Tuberculous proteins lose potency if exposed to light; consequently, large quantities are dispensed in amber-colored glass containers. Do not store tuberculin in clear syringes unless they are placed in a dark container.
- Caudal fold tuberculin (in 1cc, 5cc, and 10cc amber-colored glass containers) is to be properly discarded 3 months after initial use. Comparative cervical paired tuberculin and cervical tuberculin (1cc labeled glass containers) are to be properly discarded after use at the end of each day.
- Comparative cervical tuberculin is to be used as matching pairs only. Check serial numbers on the labels for verification.
- Do not store tuberculin in dose amounts for longer than 12 hours. Tuberculous protein will adhere to the inner surfaces of containers. Small amounts of tuberculin stored in containers with a large surface area can lose almost 100 percent of their potency.

8.4.1.5 Materials and equipment for conducting official bovine tuberculosis skin tests

- The following materials and equipment are needed to properly conduct official bovine tuberculosis skin tests:
 - USDA VS supplied PPD Tuberculin.
 - Syringe: 1.0 cc and/or 0.5 cc disposable plastic or glass tuberculin syringes.
 - **NOTE:** The use of multi-dose syringes is strongly discouraged. Use of a multidose syringe for bovine tuberculosis skin testing requires prior written approval by the AD and State or Tribal Veterinarian.
 - Needles: 26 gauge, 1 centimeter (3/8 inch) long (the only permitted size). Use a new needle for each animal.
 - **NOTE:** Deviations from this policy require prior written approval by the AD and State or Tribal Veterinarian and a signed written approval by the owner of the animals.
 - Absorbent cotton or paper towels.
 - Isopropyl alcohol (70 percent).
 - Lariat, halter, chute, head gate, and/or other equipment for restraint.
 - Official identification devices and applicators.
 - VS Form 6-22, "Tuberculin Test Record," or State equivalent; VS Form 6-22B, "Tuberculin Test Record Continuation Sheet"; VS Form 6-22C, "Tuberculin Test Record (Special)"; VS Form 6-22D, "Comparative Cervical Tuberculin Test Results", as appropriate.
 - Hair clipper with a Number 40 size blade (or equivalent)
 - USDA VS supplied dermal thickness gauge (calipers) for the CCT.
 - Other equipment, as deemed necessary, to ensure safe and efficient completion of testing (e.g., flashlight, protective equipment, bucket, brush, disinfectant, rubber boots, soap).

8.4.1.6 General injection techniques for conducting official bovine tuberculosis skin tests

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- Step 1: Ensure the injection site is free of manure, debris, and hair. Clean the site using soapy water or alcohol with cotton, paper, or other suitable material. Clip hair for the CT and CCT.
- Step 2: Check the syringe for cleanliness and leakage before using.
- Step 3: Use a new 26 gauge, 1 centimeter (3/8 inch) length needle for each animal to minimize transmission of blood-borne, infectious agents between animals.
- Step 4: Ensure that the injection of tuberculin is intradermal. To do this, 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.
- 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 of the animal. 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.
- Do not inoculate the animal being tested with vaccines or administer drugs, pharmaceuticals, or anthelmintics in conjunction with tuberculin injections. 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 skin test. Such products should be administered at the time of observation rather than at the time of injection.

8.4.1.7 Determining results of official bovine tuberculosis skin tests ("reading" the test)

- Observe and palpate the injection site at 72 (\pm 6) hours post-injection for all cattle, bison, and cervids tested. Observation without palpation is not acceptable.
- The veterinarian who makes the tuberculin injection must be the one who "reads" (determines) the results of that test.
- **NOTE:** Deviations from this policy require prior written approval by the AD and State or Tribal Veterinarian.
- Verify that every animal that was injected with tuberculin was presented for observation and palpation by checking animal identification.
- Consider ANY increase in caudal fold thickness at the site of injection -- either observed or palpated -- a positive test result (i.e., a response).
- Classify the animal with a positive test result (i.e., a response) as a suspect, unless the reactor classification is indicated.

- **IMMEDIATELY report ALL positive test results (i.e., responses) to the State or Tribal Veterinarian or the AD of the State or Tribe where the animal is located.**
- Complete and submit the VS Form record test results as specified below.

8.4.2 Approval of veterinarians to conduct official bovine tuberculosis tests

8.4.2.1 Criteria for approval of Federal and State/Tribal veterinarians to conduct the CT and CCT tests

- The requesting veterinarian must:
 - Be a State, Tribal, or Federal employee.
 - Have attended a bovine tuberculosis epidemiology training course, the epidemiologist designated by the District Director training course, or other equivalent training course sponsored by VS.
 - Have received specific training in the application and interpretation of the CT and/or CCT test through a training course sponsored by VS or through training provided by a bovine tuberculosis CHG specialist.
- For those individuals conducting CCT tests in a zoological park:
 - Be employed by a zoological park that has been accredited by the American Zoo and Aquarium Association.
 - Have received special training in the application and interpretation of the CCT test by VS.
 - Be allowed to conduct CCT on only non-program species. Cattle, bison, and captive cervids must receive a CCT test administered by an approved State or Federal veterinarian.
 - Be approved by the CHG specialist.

8.4.2.2 Approval process for BTB QAVs to collect blood for the bovine gamma interferon test (BOVIGAM®)

BTB QAV for bovine tuberculosis may collect blood from cattle in States or Tribes, when approved by the State/Tribal Veterinarian and the AD.

8.4.3 Screening tests for bovine tuberculosis

8.4.3.1 Caudal fold tuberculin (CFT) test

- Test description:
 - The caudal fold tuberculin (CFT) test is an official test for bovine tuberculosis in cattle and bison.
 - The CFT test is based on observation of a delayed-type hypersensitivity (Type IV) immune response. Animals infected with or previously exposed to mycobacterial

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species exhibit an inflammatory response at the site of an intradermal injection of PPD tuberculin.

- Appropriate use of the test:
 - The CFT test is used as a screening test for bovine tuberculosis infection in cattle and bison.

- Official testers:
 - State/Tribal, Federal, or BTB QAVs for bovine tuberculosis may apply the CFT test.
 - BTB QAVs may NOT apply the CFT test in TB affected herds or herds under epidemiological investigations. Only State/Tribal or Federal regulatory veterinarians may perform the CFT test in these herds.
 - Animal health technicians employed by a State, Tribal, or Federal government may apply the CFT test as a screening test when approved to do so by such governments and directly supervised by State, Tribal, or Federal veterinarians.

- Sample type:
 - Intradermal skin test

- Performing the test:
 - Properly restrain the animal
 - The injection site for the CFT test is in the caudal fold - 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
 - Consistently use the same side of the caudal fold for all animals tested within a lot or herd. Record the side used for the injection on the test record.
 - Note any abnormalities found near the injection site on the test record so that such abnormalities will not be mistaken for tuberculin responses during test observation.
 - If necessary, the testing veterinarian may use the opposite side of the caudal fold.
 - Do not collect blood from the coccygeal vein 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.
 - Inject 0.1 ml PPD Bovine Tuberculin intradermally in the caudal fold.
 - Observe and palpate the injection site 72 (± 6) hours after injection.
 - The veterinarian who makes the tuberculin injection must be the one who “reads” (determines) the results of the test.

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- The tail must be raised sufficiently to stretch the caudal fold slightly.
- Palpate the length of the caudal fold that was injected for responses.

Figure 4 Proper location for intradermal injection of tuberculin for the CFT test



- Test interpretation and animal classification:
 - ANY increase in caudal fold thickness at the site of injection -- either observed or palpated -- is considered a positive test result (i.e., a response).
 - Classify the animal with a positive test result (i.e., a response) as a suspect, unless the reactor classification is indicated.
 - Epidemiologists designated by the District Director and CHG specialists may classify a CFT-responding animal as a reactor when that classification is indicated.
 - For example, the reactor classification may be indicated when an animal or the herd in which it resides may have been or was exposed to a bovine tuberculosis-affected herd or an animal infected with *M. bovis*.
- Specific instructions for reporting:

- **The official tester must IMMEDIATELY (same working day) report ALL positive test results (i.e., responses) to the State or Tribal Veterinarian or the AD of the State or Tribe where the animal is located.**
- Complete and submit the VS Form 6-22, “Tuberculosis Test Record”.
- For the CFT test results:
 - Record a plus (+) sign in the first column under “Results” labeled “Size” for any increase in caudal fold thickness or other inflammatory response at the site of the injection.
 - For the second column under “Results” labeled “NRS”, mark with an N (negative) when no response was detected or an S (suspect) when a (+) in the size column indicates that response did occur.
- Retesting an animal
 - Animals that are suspects on the CFT must be retested with a secondary test to determine their final classification.
 - The CFT may not be applied to bovines within 60 days of any prior tuberculin injections.

8.4.3.2 *Single cervical tuberculin (SCT) test*

- Test description:
 - The SCT test is an official test for bovine tuberculosis in cervids.
 - The SCT test is based on observation of a delayed-type hypersensitivity (Type IV) immune response. Animals infected with or previously exposed to mycobacterial species exhibit an inflammatory response at the site of an intradermal injection of PPD tuberculin.
- Appropriate use of the test:
 - The SCT is used as a screening test for bovine tuberculosis infection in cervids.
- Official testers:
 - State, Federal, or BTB QAVs for bovine tuberculosis may apply the SCT test.
 - BTB QAVs may NOT apply the SCT test in TB affected captive cervid herds or captive cervid herds under epidemiological investigations. Only State, Tribal, or Federal regulatory veterinarians may perform the SCT test in these herds.
- Sample type:
 - Intradermal skin test
- Performing the test:
 - Properly restrain the animal. Proper restraint of cervids is necessary to apply the SCT and to ensure the safety of the official tester(s).

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- The injection site for the SCT test is in the mid-cervical region.
 - Prepare the injection site by clipping a 6 centimeter (2 ½ inch) square area.
 - Note any abnormalities found near the injection site on the official test record so that such abnormalities will not be mistaken for tuberculin responses during test observation.
 - Mark the injection site before injecting by drawing a 12 mm (0.5 inch) circle with a permanent marker. This may enable the tester to find small responses when reading the test.
- Inject 0.1 ml PPD Bovine Tuberculin intradermally in the middle of the clipped area.
- Observe and palpate the injection site 72 (± 6) hours after injection.
 - Properly restrain the animal.
 - The veterinarian who makes the tuberculin injection must be the one who “reads” (determines) the results of the test.
- Grasp the skin so as to cause a fold of skin at the injection site. Palpate the injection site by running the thumb and forefinger of the opposite hand back and forth along the fold. Palpation which is limited to running the fingertips over the skin surface is not acceptable. Test interpretation and animal classification:
- ANY increase in skin thickness at the site of injection – either observed or palpated – is considered a positive test result (i.e., a response).
- Classify the animal with a positive test result (i.e., a response) as a suspect, unless the reactor classification is indicated.
 - Epidemiologists designated by the District Director or CHG specialists may classify a SCT responding animal as a reactor when that classification is indicated.
 - For example, the reactor classification may be indicated when an animal or the herd in which it resides may have been or was exposed to a bovine tuberculosis-affected herd or an animal infected with *M. bovis*.
- Specific instructions for reporting:
 - **The official tester must IMMEDIATELY (same working day) report ALL positive test results (i.e., responses) to the State/Tribal Veterinarian or the AD of the State or Tribe where the animal is located.**
 - Complete and submit the VS Form 6-22, “Tuberculosis Test Record”.
 - For the SCT test results:
 - Record the estimated increase in skin thickness (in millimeters) in the first column under “Results” labeled “Size” for any increase in caudal fold thickness or other inflammatory response at the site of the injection.
 - For the second column under “Results” labeled “NRS”, mark with an “N” (negative) when no response was detected or an “S” (suspect) when a the size column indicates that response did occur.
- Retesting an animal:
 - Animals that are suspects on the SCT must be retested with a secondary test to determine their final classification.

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- The SCT may not be applied to a captive cervid within 90 days of any prior tuberculin injections.
- Use alternating sides of the neck for subsequent tests.

8.4.3.3 Cervical tuberculin (CT) test

- Test description:
 - The cervical tuberculin (CT) test is an official test for bovine tuberculosis in cattle and bison.
 - The CT test is based on observation of a delayed-type hypersensitivity (Type IV) immune response. Animals infected with or previously exposed to mycobacterial species exhibit an inflammatory response at the site of an intradermal injection of PPD tuberculin.
- Appropriate use of the test:
 - The CT is a screening test that is limited to use only in cattle and bison known to have been exposed to *M. bovis*.
 - The CT is the only test approved for use on exposed animals acquired from a bovine tuberculosis-affected herd (VS Form 6-4b investigations).
 - The test may also be used for testing bovine tuberculosis-affected herds managed under a test-and-remove protocol.
 - The CHG specialist may recommend the use of the CT in specific animals or herds under investigation.
- Official testers:
 - Only approved State, Tribal, or Federal veterinarians may apply the CT test.
- Sample type:
 - Intradermal skin test
- Performing the test:
 - Properly restrain the animal.
 - The injection site for the CT test is in the mid-cervical region.
 - Prepare the injection site by clipping a 7.8 centimeter (3 inch) square area.
 - Note any abnormalities found near the injection site on the official test record so that such abnormalities will not be mistaken for tuberculin responses during test observation.
 - Mark the injection site before injecting by drawing a 12 mm (0.5 inch) circle with a permanent marker. This may enable the tester to find small responses when reading the test.
 - Inject 0.1 ml of bovine cervical test PPD tuberculin (PPD Bovine, Cervical, 2mg/ml) intradermally in the middle of the clipped area.
 - Observe and palpate the injection site 72 (\pm 6) hours after injection.
 - Properly restrain the animal.
 - The veterinarian who makes the tuberculin injection must be the one who “reads” (determines) the results of the test.

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- Grasp the skin so as to cause a fold of skin at the injection site. Palpate the injection site by running the thumb and forefinger of the opposite hand back and forth along the fold. Palpation which is limited to running the fingertips over the skin surface is not acceptable.

Figure 5 Proper location and injection technique for the CT in cattle and bison



- Test interpretation and animal classification:
 - ANY detectable change, palpable or visual, at the site of injection -- either observed or palpated -- is considered a positive test result (i.e., a response).
 - Animals with a positive test result (i.e., a response) MUST be submitted for post-mortem evaluation.
 - Recall that this test uses a more concentrated dosage of tuberculin to increase the sensitivity of the test.
 - All responders are taken to necropsy or slaughter for further evaluation.
 - The animal may be classified as either a suspect or a reactor. Consult with the epidemiologist designated by the District Director or the CHG specialist to determine which classification is indicated.

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- Specific instructions for reporting:
 - **The official tester must IMMEDIATELY (same working day) report ALL positive test results (i.e., responses) to the State/Tribal Veterinarian or the AD of the State or Tribe where the animal is located.**
 - Complete and submit the VS Form 6-22, “Tuberculosis Test Record”.
 - For the CT test results:
 - Although the size of responses is not a factor in classification, record the estimated increase in skin thickness (in millimeters) in the first column under “Results” labeled “Size” for any increase in caudal fold thickness or other inflammatory response at the site of the injection.
 - For the second column under “Results” labeled “NRS,” mark with an “N” (negative) when no response was detected or an “R” (suspect) when a the size column indicates that a response did occur.
- Retesting an animal
 - Testing interval \geq 60 days.
 - The CT may not be applied to an animal within 60 days of any prior tuberculin injection.
 - Use alternating sides of the neck for subsequent tests.
 - Revert to using the CFT test after two consecutive CT tests fail to reveal lesioned reactors.

8.4.3.4 IDEXX *M. bovis* Ab Test

- Test description:
 - The IDEXX *M. bovis* Ab Test is an official test for bovine tuberculosis in TB affected cattle herds during the test and removal phase of a herd management plan only.
 - The IDEXX *M. bovis* Ab Test is a test for serum antibodies to *M. bovis*.
- Appropriate use of the test:
 - The IDEXX *M. bovis* Ab Test may be used during the removal phase of test-and-remove management plans in TB-affected cattle herds. Other uses will be considered on a case-by-case basis.
 - The IDEXX *M. bovis* Ab Test is approved for cattle 3 months of age and older.
 - Use of the IDEXX *M. bovis* Ab Test is at the discretion of the epidemiologist designated by the District Director or Area Epidemiology Officer (AEO) with approval required by the commodity health group specialist.
- Official testers:
 - State, Tribal, or Federal Regulatory veterinarians will collect blood samples for the IDEXX *M. bovis* Ab Test.
 - Technicians employed by a State, Tribal, or Federal government and approved by such government may collect blood for the IDEXX *M. bovis* Ab Test when directly supervised by State, Tribal, or Federal animal health veterinarians.

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- Sample type:
 - Serum
 - Blood samples for IDEXX *M. bovis* Ab Testing must be collected no sooner than 7 days after the CFT is injected and no more than 75 days after the CFT is injected. To ensure rapid identification and removal of potentially infected animals, VS recommends collecting blood samples between 7 and 14 days after the CFT is injected.
 - Attach a copy of the completed VS Form VS 6-22 (Tuberculosis Test Record).
- Test interpretation and animal classification:
 - The epidemiologist designated by the District Director will classify the animals interpreting the CFT and IDEXX *M. bovis* Ab Tests in parallel.
 - Animals negative on the CFT and IDEXX *M. bovis* Ab Test, SIP ratio < 0.3, should be classified as negative.
 - Animals nonnegative on the CFT or the IDEXX *M. bovis* Ab Test, S/P ratio \geq 0.3, must be examined postmortem for evidence of TB.

8.4.4 Secondary tests for bovine tuberculosis

8.4.4.1 Comparative cervical tuberculin (CCT) test

- Test description:
 - The comparative cervical tuberculin (CCT) test is an official test for bovine tuberculosis in cattle, bison, and cervids.
 - The CCT test is based on observation of a delayed-type hypersensitivity (Type IV) immune response. It distinguishes between cell-mediated immune responses to *M. avium* from those to *M. bovis* by comparing the animal's responses to intradermal injections of both types of PPD tuberculin.
- Appropriate use of the test:
 - The CCT is used as a secondary test for bovine tuberculosis infection in cattle, bison, and captive cervids classified as a suspect based on a positive test result (i.e. response) to a tuberculin skin test during screening.
 - This test is not to be used during the removal stage of testing in known infected *M. bovis* herds. Exceptions must be approved by the epidemiologist designated by the District Director in conjunction with the CHG specialist.
- Official testers:
 - Only approved State, Tribal, or Federal veterinarians may apply the CCT test.
- Sample type:
 - Intradermal skin test
- Performing the test:
 - Properly restrain the animal.

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- The injection sites for the CT test are in the mid-cervical region.
 - Prepare two injection sites. 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.
 - Clip an 8 centimeter (3 inch) square area at each site.
 - Prior to injection, lift a fold of skin at the center of each clipped site and measure the fold to the nearest 0.5 millimeters with approved calipers.
 - Record the measurements on VS Form 6-22C, “Tuberculin Test Record (Special)”, as described below.
- Inject the balanced PPD tuberculins intradermally as follows:
 - 0.1 ml PPD avian in the upper site
 - 0.1 ml PPD bovine in the lower site.
 - Inject the test sites in the same order on each animal to reduce interpretation errors. (Remember: avium over bovine)
 - Use separate syringes and needles for each type of tuberculin to ensure that the avian and bovine tuberculins never mix.
 - Use identical types of syringes and needles for each tuberculin injection.
- Palpate the injection site and measure skin thickness 72 (\pm 6) hours after injection.
 - The veterinarian who makes the tuberculin injection must be the one who performs the pre- and post-injection measurements and “reads” (determines) the results of the test.

NOTE: If a deviation from this policy was approved in advance and documented in writing by the AD and State Veterinarian, the test may be read by another regulatory veterinarian who is approved to conduct the CCT. In this situation, the pre-injection measurements may be of non-affected skin adjacent to the response.

- 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 approved calipers used to measure the normal skin.
- Test interpretation and animal classification:
 - The goal of interpretation at this stage of testing is to determine the likelihood that a positive result on a skin test (i.e., a response) in an individual animal is the result of infection with *M. bovis*. The interpretations of the results of the CCT test should consider pertinent herd and animal history, as well as presumptive test results for the animal.

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- Complete and submit the VS Form 6-22C, “Tuberculin Test Record (Special).” For both the avium and bovine PPD injection sites:
 - Record pre- and post-injection measurements to the nearest 0.5 mm in columns labeled “Normal” and “72 Hours.”
 - Record the difference after subtracting the pre-injection measurement from the post-injection measurement in the column labeled “increase.”
 - Complete and submit the VS Form 6-22D, “Comparative Cervical Tuberculin Test Results.”
 - Plot a single point representing the skin thickness difference value for both the avian (y-axis) and bovine (x-axis) PPD tuberculin injection sites for a single animal.
 - Use completed form VS Form 6-22D, Comparative Cervical Tuberculin Test Results (i.e., the scattergram) as a basis to classify the animal.
 - In general, classify each animal according to the zone into which its test results are plotted on the scattergram: negative for *M. bovis* (N), suspect (S), or reactor (R).
 - Use the bovine boundary lines to classify reindeer tested with the CCT.
 - Use the more severe category of classification for animals with a test result that is plotted on a boundary line on the scattergram.
 - Record the classification of the animal on the VS Form 6-22C as:
 - “N” for negative *M. bovis*
 - “S” for suspect
 - “R” for reactor
 - The following information about the herd or animal bovine tuberculosis testing history will influence the interpretations of the results of the CCT test and the classification of the animal.
 - Classify the following animals as reactors:
 - Animals with test results that plotted in the reactor zone on any CCT tests.
 - Animals with test results that plotted in the suspect zone on two successive CCT tests.
 - Animals that responded to the screening test and were subsequently found to be negative or suspect to the CCT test if *M. bovis* infection is confirmed in the herd.
 - The epidemiologist designated by the District Director or CHG specialist may reclassify an animal responding in the reactor zone, or responding twice in the suspect zone, as a suspect.
 - Animals classified as a suspect MAY be submitted for post mortem evaluation.
 - Animals reclassified as a suspect by the epidemiologist designated by the District Director or the CHG specialist MUST be submitted for post mortem evaluation.
 - Animals classified as a reactor MUST be submitted for post mortem evaluation.
- Specific instructions for reporting
 - **The official tester must IMMEDIATELY (same business day) report ALL positive test results (i.e., responses) to the State/Tribal Veterinarian or the AD of the State or Tribe where the animal is located.**

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- Retesting an animal
 - If used in cattle or bison, the CCT test must be applied within 10 days of the injection date of a CFT test. Otherwise, it must be administered more than 60 days after the date of injection of a CFT test with a positive test result (i.e., a response).
 - If used in cervids, the CCT test must be applied within 10 days of the injection date of a SCT test. Otherwise, it must be administered more than 90 days after the date of injection of a SCT test with a positive test result (i.e., a response).
 - Use the opposite side of the neck from SCT test injection, especially if the two tests are applied within 10 days of each other.
 - Testing interval ≥ 60 days in cattle and bison; ≥ 90 days in cervids.
 - The CCT test may not be applied to cattle or bison within 60 days of any prior tuberculin injection in cattle or bison or within 90 days for cervids.
 - An animal classified as suspect on the basis of the CCT test may be retested once with the CCT. The second CCT test must occur no sooner than 60 days after the first CCT test injection in cattle and bison and 90 days after the first CCT test injection in cervids.
 - An animal that plots in the suspect zone on two consecutive CCT tests should be classified as a reactor (R), unless classified as a suspect by the epidemiologist designated by the District Director or the CHG specialist, provided that there has been no known association of the herd with *M. bovis*.

8.4.4.2 Bovine gamma interferon test

- Test description:
 - The bovine gamma interferon test (BOVIGAM[®]) is an official test for bovine tuberculosis in cattle greater than 6 months of age.
 - The bovine gamma interferon test (BOVIGAM[®]) distinguishes between cell-mediated immune responses to *M. avium* from those to *M. bovis* by comparing the relative amount of interferon gamma that an animal's macrophages produce in response to both types of PPD tuberculin in an ELISA format.
 - Cattle 6 months of age and younger should not be tested using the gamma interferon test because non-specific reactivity to bovine tuberculin occurs, resulting in false positive responses.
- Appropriate use of the test:
 - The bovine gamma interferon test (BOVIGAM[®]) is used as a secondary test for bovine tuberculosis infection in cattle classified as a suspect based on a positive test result (i.e. response) to a tuberculin skin test during screening.
 - The State or Tribal Veterinarian and the AD must approve the use of the bovine gamma interferon test (BOVIGAM[®]) as a replacement for the CCT.
 - The bovine gamma interferon test (BOVIGAM[®]) is NOT approved for official use in other species.
 - The bovine gamma interferon test (BOVIGAM[®]) is NOT approved for use as a screening test.
 - The bovine gamma interferon test (BOVIGAM[®]) may also be used in parallel with the CFT test in bovine tuberculosis-affected herds that are managed under a test-and-

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removal herd plan at the discretion of the epidemiologist designated by the District Director with the concurrence of the CHG specialist.

- Official testers:
 - Approved State/Tribal or Federal veterinarians may collect blood from cattle.
 - Approved animal health technicians employed by a State, Tribal, or Federal government may collect blood from cattle when approved to do so by such governments and directly supervised by State, Tribal, or Federal veterinarians that are on-site at the time of testing.
 - BTB QAVs for bovine tuberculosis may collect blood from cattle in States or Tribes, when approved by the State or Tribal Veterinarian and the AD.
 - Only approved laboratories that utilize standard operating procedures approved by NVSL may perform the bovine gamma interferon test (BOVIGAM®) as an official test for bovine tuberculosis in cattle.

- Sample type and collection:
 - Collect 6 ml or more of whole blood in a heparinized (i.e., green-topped) blood tube. Other anti-coagulants interfere with this test.
 - Collect blood 3 to 30 days after CFT injection from CFT test responders (i.e. animals classified as suspects) at the time the CFT test is read. It is critical to maintain blood at at $22^{\circ}\pm 5^{\circ}$ C ($71.6^{\circ}\pm 9^{\circ}$ F) during handling and shipping. Contact the approved laboratory or NVSL for specific shipping instructions.

- Test interpretation:
 - **Negative:** Samples with a negative result are reported as being negative for *Mycobacterium bovis*. A sample is considered negative if the OD value of either the nil antigen or the avian antigen subtracted from the OD value of the bovine antigen gives a value less than 0.1. Negative = bovine antigen OD - nil antigen OD < 0.1 or bovine antigen OD – avian antigen OD < 0.1
 - **Positive:** Samples with a positive result are reported as being positive for *Mycobacterium bovis*. A sample is considered positive if the OD value of the nil antigen subtracted from the OD value of the bovine antigen gives a value greater than or equal to 0.1, and the OD value of the avian antigen subtracted from the OD value of the bovine antigen gives a value greater than or equal to 0.1. Positive = bovine antigen OD – nil antigen OD \geq 0.1 and bovine antigen OD – avian antigen OD \geq 0.1
 - **Nonviable:** Nonviable samples are reported as being nonviable. For a sample to be considered viable, the OD value of the pokeweed mitogen well for the sample must be at least 0.1 greater than the OD value of the nil antigen well for that sample. Valid sample = pokeweed OD \geq nil antigen OD + 0.1
 - **Not Tested:** Sample did not meet previously described criteria for testing.

- Animal classification:
 - The epidemiologist designated by the District Director, in consultation with the CHG specialist, interprets the test results described to classify the animal.

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- Generally, an animal with a positive result on a routine test for sale, show, movement, or milk ordinance in AF zones, the bovine gamma interferon test (BOVIGAM®) (where positive and negative controls meet requirements) are classified as:
 - An animal with a positive result where bovine antigen OD – avian antigen OD is between 0.1 and 0.4999 should be classified as a suspect.
 - An animal with a positive result where bovine antigen OD – avian antigen OD is ≥ 0.5000 should be classified as a reactor. The epidemiologist designated by the District Director or the CHG specialist may reclassify the animal as suspect but the animal must be evaluated for tuberculosis postmortem.
- If the initial gamma interferon test is not tested or nonviable:
 - Test with the CCT, if still prior to the 10 day window from the CFT, and use the CCT results as the official results, or:
 - Retest with the gamma interferon if past the 10 day window and before 30 days from the CFT.
- If the gamma interferon test is not tested or nonviable twice, the animal remains a suspect and may be retested with CCT after waiting 60 days from the CFT.
- Information about the herd's or animal's bovine tuberculosis testing history and other risk factors may influence the interpretation of the test results and the classification of the animal.
 - Animals with positive test results on two successive gamma interferon tests should be classified as a reactor. However, the epidemiologist designated by the District Director or the CHG specialist may reclassify this animal as a suspect, if indicated, but it must be evaluated postmortem.
 - The epidemiologist designated by the District Director or the CHG specialist may classify an animal as a suspect based on a different cut-off value for a positive result, when deemed appropriate.
 - The epidemiologist designated by the District Director or the CHG specialist may classify an animal as a reactor based on a relatively high OD value or based on a positive test result in light of herd or animal test history.
- Specific instructions for reporting:
 - **The official tester must IMMEDIATELY (same working day) report ALL positive test results to the State/Tribal Veterinarian or the AD of the State or Tribe where the animal is located.**
- Retesting an animal:
 - The bovine gamma interferon test (BOVIGAM®) must only be used for blood samples collected between 3 and 30 days after the injection date of a CFT test.
 - The epidemiologist designated by the District Director or the CHG specialist may approve a retest for animals with a positive result on one bovine gamma interferon test (BOVIGAM®) so long as the blood sample for the second test is collected within 30 days of the injection date of a CFT test.

8.4.4.3 *Reclassification of CCT and Gamma interferon suspects as negative*

- CCT test suspects must be retested negative by the CCT test prior to reclassifying such suspects as negative.

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- Gamma interferon suspects must be retested negative by the gamma interferon test prior to reclassifying such suspects as negative.
- Gamma interferon suspects may not be retested with the CCT.
- CCT suspects may not be retested with the gamma interferon.
- When both CCT and Gamma interferon are used on the same CFT suspect animal, they must be interpreted in parallel.

8.4.5 CervidTB DPP test

- Test description:
 - The CervidTB DPP is an official test for bovine tuberculosis in elk, red deer, white-tailed deer, fallow deer, and reindeer.
 - The CervidTB DPP is a serological test that tests test for antibodies to TB in the serum
- Appropriate use of the test:
 - The DPP test is used both as a screening and a secondary test for bovine tuberculosis infection in the approved cervid species.
 - The CervidTB DPP may be used in parallel with the SCT and CCT tests but a positive DPP or CCT test will classify the animal as a suspect.
- Official testers:
 - Approved State/Tribal or Federal veterinarians may collect blood from cervids.
 - Approved animal health technicians employed by a State, Tribal, or Federal government may collect blood from cervids when approved to do so by such governments and directly supervised by State, Tribal, or Federal veterinarians that are on-site at the time of testing.
 - BTB QAVs for cervid tuberculosis may collect blood from approved cervids.
 - Only approved laboratories that utilize standard operating procedures approved by NVSL may perform the DPP as official test for bovine tuberculosis in approved cervids.
 - Include a copy of the VS Form 6-22 including all the tube numbers and animal ID's may also be included with the VS 10-4 to provide the tube numbers and animal ID's.
- Sample testing
 - Valid serum samples will be tested using the DPP test following test kit instructions and internal NVSL standard operating procedures.
 - If the DPP primary test result obtained is above the optical density reader value cutoff point that has been established, the test will be called positive.

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- Results of the DPP primary test will be reported to the submitting accredited veterinarian, the respective State animal health official, Assistant District Director (ADD), and district or designated epidemiologist of the State where the animals are located. The district or designated epidemiologist will report to the CHG cervid staff epidemiologist.
- The District Epidemiologist or CHG cervid staff epidemiologist will classify the animals.
 - Animals negative on the DPP primary test should be classified as negative.
 - Animals non-negative on the DPP primary test should be classified as suspect unless the District or CHG cervid staff epidemiologist determines that a reactor classification is warranted.
 - Animals classified as suspect by a DPP primary test may be retested with the DPP test with a new blood sample drawn no sooner than 30 days after the initial sample was obtained.
 - Animals testing negative on the second DPP test should be classified as negative.
 - Animals that are non-negative on two successive DPP tests should be classified as reactor.
- Any exceptions to reactor classification must be justified by the District epidemiologist in writing and have the concurrence of the Cervid Health staff epidemiologist.
- The animal is then handled according to its classification following TB program regulations.
- TB Program Testing Protocol
 - If an animal tests non-negative to the primary DPP serological test, it must be retested in not less than 30 days using the DPP serological test as the secondary test.
 - If an animal tests non-negative to the SCT, it must be retested using the comparative cervical tuberculin skin test (CCT) as the secondary test.
 - The CCT will not be used as a primary test for any animals.
 - If parallel testing is performed with the DPP and SCT tests, it must be completed with permission from and consultation with the District epidemiologist and the CHG cervid staff epidemiologist. The testing protocol, timing of different tests, interpretation of the tests, classification of the animals, and disposition of the animals must be determined in the protocol before the testing. Secondary tests to any non-negative primary test must follow the first two instructions above.
 - In routine herd testing, different groups of animals within a herd may be tested using the different methods (i.e., bucks tested serologically and does tested via the skin test) if a different VS Form 6-22 is used for each group of animals. However, individual animals testing non-negative to a primary test must be followed up with a test of the same test method as required in the first two instructions above.
 - In affected herds or herds under investigation, a testing protocol using serological and skin tests separately or in series or parallel may be devised and used by the

designated or District epidemiologist and CHG cervid staff epidemiologist in consultation.

8.4.6 Post-mortem tests for bovine tuberculosis

8.4.6.1 Test description

- A combination of histopathology, mycobacterial culture, PCR, and/or genotyping is used to further evaluate tissues collected during necropsy or at the time of slaughter for the presence of *M. bovis*.
 - **Histopathology** is a rapid method of identifying structural changes in tissues associated with mycobacterial infections. Mycobacteriosis compatible: This diagnosis means that the lesion is consistent with tuberculosis and the granuloma contains acid-fast bacteria. Because the species of acid-fast bacteria causing this lesion cannot be determined using histopathology alone, a diagnosis of mycobacteriosis compatible is not a diagnosis of infection with *M. bovis*.
 - **PCR**
 - May be performed after a mycobacteriosis compatible diagnosis on formalin fixed paraffin embedded (FFPE) tissue and is used to determine the presence of genetic material from the *Mycobacterium tuberculosis* complex (which includes *M. bovis*, *M. tuberculosis*, and several other species), *M. avium* and *M. a. paratuberculosis* in tissue.
 - May be performed on grossly lesioned fresh or borate tissue. PCR may be used in place of culture for routine slaughter surveillance samples. Program staff must approve the use of PCR in place of culture for all other cases. PCR is not an appropriate test for grossly normal tissue.
 - **Mycobacteriologic culture** isolates mycobacteria from the tissue and the permits definitive identification and genotyping of *M. bovis*.
 - **Genotyping** using whole genome sequencing is a high resolution method for determining the relatedness of isolates.

8.4.6.2 Appropriate use of the tests

- A combination of histopathology, mycobacterial culture, genotyping, and/or PCR testing is used as the final stage of testing for bovine tuberculosis and permits the final classification of suspect, reactor, and exposed animals as infected with *M. bovis* or negative.
- Animals that have acid fast bacteria recovered on culture that are speciated as *M. bovis* are confirmed tuberculosis-infected.
- Animals that have mycobacteriosis compatible lesions or are epidemiologically linked to *M. bovis* cases and are also PCR-positive for *Mycobacterium tuberculosis* complex will be classified as confirmed tuberculosis-infected.

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8.4.6.3 Official testers

- *Sample collection at slaughter of regular kill animals.* FSIS food inspectors and public health veterinarians may collect granulomatous lesions detected at the time of routine post-mortem slaughter inspection of regular-kill animals.
- *Sample collection at slaughter of bovine tuberculosis reactors, -suspects, or -exposed animals.* State, Tribal or Federal veterinarians may conduct post-mortem procedures and collect tissues from animals that are classified as bovine tuberculosis reactors, -suspects or -exposed.
- *Sample collection at the time of on-farm necropsy of bovine tuberculosis reactors, -suspects, or -exposed animals* State, Tribal or Federal veterinarians may conduct necropsies and collect tissues from animals that are classified as bovine tuberculosis reactors, -suspects or -exposed.
- Approved animal health technicians employed by a State, Tribal, or Federal government may collect tissues when approved to do so by such governments and directly supervised by State, Tribal, or Federal veterinarians.

8.4.6.4 Sample collection at slaughter of regular kill animals

Refer to [FSIS Directive 6240.1](#) for detailed instructions concerning the inspection and collection of tissue samples at the time of slaughter.

8.4.6.5 Sample collection at slaughter: Bovine tuberculosis-reactors, -suspects, or -exposed animals

State and federal animal health officials should complete the following actions when bovine tuberculosis-reactor, -suspect, or -exposed animals are to be presented for slaughter:

- Identify animals, complete VS Form 1-27, Permit for Movement of Restricted Animals, and seal truck, as described in Appendix D of the [National Veterinary Accreditation Program Reference Guide](#).
- Categorize bovine tuberculosis-exposed animals as either:

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- Category 1: Diagnostic exposed animals
 - These are animals that have been moved from a bovine tuberculosis-affected 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.
 - FSIS inspectors will perform a modified expanded post-mortem inspection procedure on these animals.
 - Category 2: Animals that are part of a known affected herd.
 - These are test negative or untested animals which may move to slaughter as regular culls or by entire herd.
 - FSIS inspectors will perform a regular post-mortem inspection procedure on these animals.
- The AD in the State or Tribe of origin should alert the AD in the State or Tribe where the animals are to be slaughtered of the pending shipment.
 - The AD in the State or Tribe where the animals are to be slaughtered should inform FSIS personnel at the receiving establishment prior to the animals' arrival.
 - VS or State/Tribal animal health personnel should be present to oversee and assist with the collection of tissue specimens as described in [FSIS Directive 6240.1](#).
 - Submit specimens collected from bovine tuberculosis-reactors, -suspects, or -exposed animals at slaughter to NVSL on a 10-4 form, noting if carcasses are retained.

8.4.6.6.1 Materials and equipment

Sampling and Shipping Supplies

The NVSL will provide the appropriate sampling and shipping supplies upon request. Requests can be emailed to NCAH.Shipping@aphis.usda.gov. The supplies needed to sample one animal include:

- For fixed tissues, at least 3 jars (90 mL) of 10% neutral buffered formalin.
- For fresh tissues, either at least 3 plastic sample bags per animal or at least three 4-ounce jars of saturated sodium borate solution.
- 8 barcodes: 3 for the buffered formalin jars (head and chest, abdomen), 3 for the plastic bags or sodium borate jars, 1 for the form and 1 red top tube for blood/serum (if applicable)
- FedEx shipping label
- Form 10-4
- Form 10-7

Necropsy Tools

Suggested necropsy supplies include:

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- Sharp knife (hunting knives and slaughterhouse boning knives work well)
- Scissors
- Rat-tooth forceps
- Cutting board
- Small and large shears (lopping shears or ratcheted rib cutters for rib cage/sternum)
- Scalpels (disposable scalpels are highly recommended)

Other Supplies

Other suggested supplies include:

- Disinfectant
- Scrub brush
- Large rubber tub (for disinfecting boots and necropsy tools)
- Datasheets
- Plastic bags (large for carcass disposal and small for sample collection)
- Sharpie® or indelible marker (for labeling)
- Pen (for filling out datasheets)
- Plastic sheets, wood chips, pet litter, or other absorbents (for floors in work area)
- Sharps container
- Biohazard waste bag
- Digital camera
- Ruler (for measuring lesions and/or tissue)

8.4.6.6.2 Personal Safety Guidelines and Equipment

Bovine tuberculosis is zoonotic and presents a risk to human health and safety. Because of this risk, all carcasses should be handled with caution and considered potentially infectious. Precautions for personal safety should be exercised.

Do not eat, drink, or smoke while dissecting a carcass or collecting samples. Establish a clean work zone and a contaminated work zone (clean/dirty line) with an area to disinfect supplies, equipment, and personnel between the two areas. Place datasheets, camera, and other non-disposable equipment in plastic bags or containers that can be disinfected or discarded.

All personnel conducting necropsies or handling animals that may be infected with bovine tuberculosis should have a bovine tuberculosis test prior to any potential exposure and annually thereafter (or as recommended by an occupational health professional).

Safety and Personal Protective Equipment (PPE)

Wearing protective gear will minimize the possibility of contact with infectious agents in body fluids and aerosols and reduce the risk of human infection. All necropsy tools and instruments should be disinfected before and between necropsies, and after sampling to prevent cross-contamination and infection.

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The following PPE are recommended during sample collection:

- Heavy-duty disposable gloves (rubber or nitrile)
- Cut-resistant mesh glove on non-dominant hand
- Goggles, safety glasses, or face shield
- Disposable apron or apron that can be disinfected
- Forearm protectors
- Cloth or Tyvek® coveralls
- Rubber boots
- Hair net or hat that can be disinfected
- Respirator (N95 mask at a minimum)

Work upwind of carcasses when performing necropsies outdoors. Always wash hands and exposed skin with soap and warm water or an alcohol based cleanser after collecting samples.

Handling Harmful Substances

Sodium borate and 10% buffered formalin are hazardous substances that can be inhaled or absorbed through the skin. Carefully handle all harmful substances when sampling and shipping.

Disinfectants are also potentially hazardous and should be handled with care. The Material Safety Data Sheets for each chemical should be reviewed prior to use to ensure that collectors are aware of the dangers associated with handling the disinfectants and chemicals and take the appropriate precautions.

Carcass Disposal and Disinfectants

The carcass and all tissues from the carcass should be disposed of according to State, Tribal and local animal carcass disposal regulations. Depending on the State, Tribe, or area, methods may include burial, incineration, composting or double-bagging and transporting to a landfill. All contaminated paper or plastic materials should be considered hazardous waste and should be thoroughly disinfected, incinerated or double-bagged and disposed of at the landfill (if permitted).

All blood and tissue should be removed from necropsy instruments and tools with soap and water, rinsed, and subsequently disinfected with an approved disinfectant for bovine tuberculosis between necropsies. If disinfectants are not used between animals, false positives may be identified.

Gloves also should be changed between animals. Necropsy boots, aprons and contaminated clothing should be cleaned and thoroughly disinfected upon completion of sample collections. External surfaces of containers with samples should be disinfected.

The products listed in the table below are effective, environmentally friendly disinfectants for use against bovine tuberculosis. Additional approved bovine tuberculosis disinfectants can be found in the U.S. Environmental Protection Agency Office of Pesticide Programs List B: EPA's Registered Tuberculocide Products Effective Against *Mycobacterium tuberculosis*.

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Disinfectant	Time to Effectively Disinfect	Environmentally Friendly	Manufacturer
Oxivir bovine tuberculosis	5 minutes	Active ingredients break down to water and oxygen	Johnson Diversey
Opti-Cide 3	3 minutes	Contains no dangerous phenols, chlorine, artificial dyes or perfumes	Micro-Scientific Industries
Clorox Bleach (Mix 1 part bleach with 9 parts water)	5 minutes	Product contains no free chlorine and breaks down into salt and water after use; does not contain dioxins or contaminate groundwater	Clorox Company

Disposal of Gloves and Sample-Related Waste

Spray or soak waste with disinfectant and place in bag. Then spray or soak bag with disinfectant, place in another bag, and dispose at the landfill (if permitted).

8.4.6.6.3 Collecting Specimens

General Recommendations

Despite the stringent decontamination protocols used in the laboratory, tissue specimens can still be overgrown by environmental fungi and bacteria, thereby impeding the ability to recover any viable mycobacteria present in the tissues. To minimize overgrowth, it is important to collect tissues from the animal as soon as possible post mortem. Collect tissues from animals within 2 hours whenever possible. When performing the necropsy, collect the tissues using aseptic techniques from the head, thoracic cavity and abdominal cavity (in that order) to minimize cross-contamination.

Samples to Collect

In summary, representative lymph nodes from the head, thorax, and abdomen as well as any lesions are collected in both formalin for histopathology and either in sodium borate jars or plastic bags for fresh chilled samples used for culture on all animals. Beginning with the head, the medial and lateral retropharyngeal lymph nodes, mandibular lymph nodes, and parotid lymph nodes are incised, examined, and subsampled for both histopathology and culture. The subsamples are placed in a formalin jar labeled “head” and a sodium borate/plastic bag for culture also labeled “head”. Next the thoracic lymph nodes including the tracheobronchial and mediastinal lymph nodes are incised, examined, and subsampled for both histopathology and culture. The subsamples are placed in a formalin jar labeled “thorax” and a sodium borate/plastic bag for culture also labeled “thorax”. The abdominal lymph nodes including the mesenteric and hepatic lymph nodes are incised, examined, and subsampled for both histopathology and culture.

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The subsamples are placed in a formalin jar labeled “abdomen” and a sodium borate/plastic bag for culture also labeled “abdomen”. If granulomatous lesions in any of these lymph nodes are identified, ideally they should be placed in a separate formalin jar and sodium borate/plastic bag labeled with the name of the lymph node and “lesion”. The following tissues are examined, but only subsampled and submitted to the laboratory if there are granulomatous lesions identified: Lung, pleura, liver, spleen, female reproductive organs, pre-scapular lymph nodes, cervical lymph nodes, popliteal lymph nodes, mammary lymph nodes, iliac lymph nodes.

All animal IDs should be collected with at least a dime size piece of tissue left associated with the identification devices. For backtags, removing the tag by pulling off the hide allowing the hair roots to remain attached to the tags is desirable.

Blood for serum should also be collected from animal exhibiting gross lesions suggestive of bovine tuberculosis.

Additional guidance on subsampling of tissue and tissue preservation

4. Always clean and disinfect instruments between necropsies of each animal. If disposable scalpels are available, discard them after each animal. Thoroughly rinse instruments after disinfecting to ensure that the samples are not inadvertently disinfected. Change gloves between the necropsy of each animal.
5. Remove excess fat from tissue samples.
6. If there are lesions identified which are too small to subdivide for both histopathology and culture, submit the small lesion only for histopathology.

Tissue preservation

Tissue Preservation Method	Test Type	Situation
Formalin	Histopathology	All instances
Sodium borate	Culture	Preferred if 10 or fewer animals are sampled
Refrigerated or Frozen in leak-proof bags	Culture	For large number of samples or for low priority research.

- Samples submitted for histopathology should be placed in 10% buffered formalin jars. Use a 10:1 formalin to tissue ratio when submitting samples for histopathology. Tissues placed in formalin should be bread sliced so that they are approximately 1 cm or less thick (e.g., the width of a pencil).
- For bacteriologic examination -- cut tissues approximately 2 to 5 cm thick and place in a sodium borate solution at a 1:1 tissue to preservative ratio.

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- Do not make additional incisions into the sample because sodium borate is bacteriocidal.
- Sodium borate solution is a supersaturated solution. It is normal to see crystals in the bottles containing the solution.

Sodium borate is generally preferred over fresh/frozen when submitting less than 10 animals for culture. Use equal parts of tissue and sodium borate (if appropriate) when submitting for mycobacteriologic culture. An advantage of sodium borate is that the tissue can be preserved without refrigeration, and it decreases the risk of tissue contamination by other bacteria. However, if the sample does not arrive at the lab for culture testing within 72 hours, the sodium borate may penetrate the tissue sample and kill any mycobacteria that may be present, increasing the risk of a false negative culture result.

For large culture submissions and lower priority research or surveillance projects, samples submitted for culture should be placed in leak proof plastic bags. Refrigerate or place in a cooler with cold packs until shipped to the laboratory. If fresh tissues for culture will be held more than 72 hours prior to shipping, freeze at -20°C and ship the tissues frozen on ice packs. Note do not freeze formalin fixed tissues.

- Tighten the caps on specimen containers and seal with Parafilm®. Electrical tape also can be used if Parafilm® is not available.
- After sample collection, disinfect the outside of each plastic bag or container in an approved disinfectant for bovine tuberculosis. Use caution to ensure that no disinfectant enters the plastic bag or the sodium borate container. Remember to keep the surface wet with disinfectant solution for the required contact time (see the previous table or refer to the product label). Rinse with water after the contact time requirement has been met.

8.4.6.6.4. Completion of submission forms

- [Forms and detailed instructions](#) are available for download.
- For samples submitted from routine postmortem examination at slaughter
 - Submit VS Form 6-35.
- For all bovine tuberculosis-reactor, -suspect, or -exposed animals
 - Submit VS Form 10-4 AND the supplemental tuberculosis collection form VS 10-7.
 - Provide all available information (owner, material submitted, animal identification, examination requested).
 - A 10-4 form must accompany each shipment and can include multiple animals.
 - Fill out a separate supplemental 10-7 for each individual animal.
 - An accurate description of the lesions is very important (size, color, location, and consistency) as is information on the type of carcass (species, sex, age, condition).
 - Be certain the animal identification on the forms correlates with the animal identification on the sample containers.

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8.4.6.6.5 *Packaging samples for shipment to NVS*

- Send all of tissues for each animal in a single shipping container overnight.
- Seal a copy of the completed VS Form 10-4 and the supplemental VS Form 10-7 form in a plastic bag and place them between the Styrofoam™ cooler and the cardboard box. Do not place forms inside the Styrofoam™ cooler.

8.4.6.6.6 *Communication when shipping samples to NVSL*

Contact the laboratory if planning on submitting a large number of animals (>10) or with any questions. The laboratory may be contacted by email at NvslCaseCoordinator@aphis.usda.gov or by phone:

- Histopathology: (515) 337-7521
- Mycobacteriology: (515) 337-7388
- Serology: (515) 337-7565

8.4.6.6.7 *Specimens from reactor swine*

Tissue samples from swine that are tested and classified as reactors as part of an investigation should be submitted when deemed necessary.

8.4.6.6.8 *Specimens from other species*

- It is the policy of VS to provide diagnostic assistance for other species of animals (from zoos, animal compounds, primate centers, roadside parks, etc.) when deemed necessary by the AD. For guidance regarding on sampling of other species, contact the appropriate bovine tuberculosis epidemiologist designated by the District Director or CHG specialist.
- NVSL will charge a user fee for avian, dogs, cats, nonhuman primates, reptiles, amphibians, environmental samples, and elephant trunk washes.
- No charge will be made for other zoo mammals when *M. bovis* is suspected.
- If submitters need to open an NVSL account, they can call 515-663-7571, User Fee Help Line.

8.4.6.6.9 *Specimens from special studies*

- Specimens from special studies must be approved in advance by NVSL and commodity center staff.
- Contact the appropriate bovine tuberculosis epidemiologist designated by the District Director or commodity center specialist for guidance regarding the approval process.

8.4.7 Supplemental tests for bovine tuberculosis

8.4.7.1. Tissue matching (*microsatellite genotyping*)

- Appropriate use of the test
 - Tissue matching (animal identification devices with the formalin fixed tissue) will be conducted on all slaughter trace (6-35) cases with IDs submitted. It will also be done on all culture, histology discrepant cases.

8.4.7.2. Blood collection for the bovine tuberculosis serum bank

- It is preferred that serum samples be collected from live animals sent for necropsy that are likely to be infected with bovine tuberculosis. Such animals include suspect or reactor animals from known bovine tuberculosis-infected herds, tracebacks from a known bovine tuberculosis-infected herd, or other animals as suggested by an epidemiologist designated by the District Director or a commodity health group specialist.
- If it is not feasible to collect blood from an animal prior to necropsy and bovine tuberculosis or tuberculosis-like granulomas or lesions are observed in the carcass, use a needle and syringe to extract as much blood as possible from the heart.
- The following is a suggested sampling protocol if serum samples are collected and submitted to the bovine tuberculosis serum bank at NVSL:
 - Place extracted blood into red-top or serum-separator tubes (collect at least three 10ml tubes, if possible, so at minimum 10mls of serum can be harvested from the blood tubes).
 - Blood should be left undisturbed for approximately 30 minutes at room temperature to encourage clot formation prior to centrifugation.
 - Centrifuge 10 minutes to separate the serum from the blood cells. (NOTE: If it is blood collected from the heart, centrifuge for 10 minutes at a minimum of 1,800 revolutions per minute to separate the serum from the blood cells. If a centrifuge is not available, serum can be obtained by placing the blood tube in a refrigerator overnight to allow the clot to form and contract.
 - Decant or transfer the serum into a new red top tube or other vial that has a screw top lid. If multiple tubes of blood have been collected, it is satisfactory to combine harvested serum into one tube (or more, if needed).
 - Identify the serum tubes in a fashion so that can be easily correlated to the animals listed on a lab submission form or a copy of the tuberculosis test chart.
 - It is preferred to maintain serum at refrigerated temperatures until the sample is shipped to NVSL.
 - Refrigerated serum should be shipped to NVSL with ice packs within 2 weeks of collection.
 - Submit, at minimum, 1 mL of serum (more if possible) to the bovine tuberculosis serum bank from each animal.
 - If it is more convenient for a facility to submit frozen serum to the bovine tuberculosis serum bank, transfer 0.5 mL of serum into as many 1.2 mL

Brucellosis and Bovine Tuberculosis Program Standards

polypropylene Cryovials® that will contain the harvested serum. Identify the tubes to be submitted in a fashion that can be easily correlated to animals listed on a lab submission form or a copy of the tuberculosis test chart. Freeze the Cryovials® at -20°C.

- Frozen serum can be shipped to NVSL once a month on dry ice to ensure the samples remain frozen during shipping. This will reduce the number of freeze-thaw cycles during processing at NVSL to ensure that sample quality is maintained.
- For all samples submitted to the bovine tuberculosis serum bank at NVSL, provide skin test and any other pre-necropsy bovine tuberculosis testing information (gamma interferon or other serology tests) to the serum bank coordinator in order to characterize the serum samples.

Appendix 1: Animal Health Plan Template

The final format for this template is yet to be determined. APHIS is exploring possibilities to allow for electronic submission. Please review content only at this time.

State/Tribe: *Select State from a pull down listing all States and select Tribes.*

Category 1: Laws and Regulations

1. Confirm that your State or Tribe has a legal and regulatory basis to conduct the activities and measures specified within this animal health plan.

Pull down menu with options:

Yes, we have the legal and regulatory authority.

No, we do not have the legal and regulatory authority.

- a. If no, please attach a document describing the amendments needed and the expected timeline to enact the necessary amendments to provide the legal and regulatory basis for the animal health plan.

Ability to attach PDF

2. Identify the law(s) and/or regulation(s) from those listed that provide your State or Tribe with the following authorities:

Authority to:	Authority exists?	Regulation Number/Citation	Title/Name
Require reporting of brucellosis (i.e., brucellosis is a reportable disease).	<i>Pull down:</i> <i>Yes</i> <i>No</i>	<i>Free text</i>	<i>Free Text;</i>
Require reporting of bovine tuberculosis (i.e., bovine tuberculosis is a reportable disease).			
Quarantine (restrict movement of) animals and herds.			
Require animal owners to present their animals for program purposes.			

3. Is the authority for the control of brucellosis delegated to a specific organization or entity within your State or Tribe? ***Pull down menu with options: Yes; No***
 - b. If yes, identify the organization or entity. ***Free text***

Brucellosis and Bovine Tuberculosis Program Standards

4. Is the authority for the control of bovine tuberculosis delegated to a specific organization or entity within your State or Tribe? **Pull down menu with options: Yes; No**
 - c. If yes, identify the organization or entity. **Free text**
5. What organization or entity within your State or Tribe has the authority to control disease in captive cervids? **Free text**
6. Are previous Federal brucellosis regulations or policies (i.e., Uniform Methods and Rules) incorporated into State or Tribal regulations? **Pull down menu with options: Yes; No**
 - d. If yes, outline the expected timeline to enact the necessary amendments.

Field to enter free text.

7. Are previous Federal bovine tuberculosis regulations or policies (i.e., Uniform Methods and Rules) incorporated into State or Tribal regulations? **Pull down menu with options: Yes; No**
 - e. If yes, outline the expected timeline to enact the necessary amendments

Field to enter free text.

Category 2: Organization and Infrastructure

8. Briefly describe the animal health and wildlife workforce within your State or Tribe that is available to implement and perform activities or maintain and enforce measures conducted under this animal health plan.

Field to enter free text.

9. How many federally accredited veterinarians practice in your State or Tribe? **Number**
10. How many Qualified Accredited Veterinarians (BTB QAVs) for bovine tuberculosis practice in your State or Tribe? **Number**

Brucellosis and Bovine Tuberculosis Program Standards

11. Complete the following table to identify the number of State, Tribal, and Federal veterinarians; animal health technicians; and wildlife biologists that conduct animal health and wildlife activities and measures carried out under this animal health plan.

Brucellosis and Bovine Tuberculosis Program Standards

Role and Activity	Number of Federal Employees	Number of State Employees	Number of Tribal Employees
Animal Health Activities			
Veterinarians	<u>Number</u>	<u>Number</u>	<u>Number</u>
Animal Health Technicians			
Wildlife Activities			
Veterinarians			
Wildlife Biologists			

12. Complete the following table to identify the designated epidemiologist(s) for both brucellosis and bovine tuberculosis in your State or Tribe.

Role	Name	Phone Number(s)	Email
Brucellosis			
Designated Epidemiologist(s)	<u>Free text</u>	<u>Numbers formatted as (XXX)-XXX-XXXX</u>	<u>Free Text;</u>
Bovine Tuberculosis			
Designated Epidemiologist(s)			

Ability for user to add rows to table as needed.

13. Are there any agreements (formal or informal) with other States, Tribes, or Federal entities that allow access to additional personnel or allow for a coordinated, joint response to any of the information categories in the animal health plan? **Pull down menu with options: Yes; No**

f. If yes, briefly describe this agreement.

<i>Field to enter free text.</i>

Brucellosis and Bovine Tuberculosis Program Standards

14. Briefly describe the diagnostic laboratory capability within your State or Tribe to support program activities and measures conducted under this animal health plan.

Field to enter free text.

15. Are there any agreements with other States, Tribes, or Federal entities that allow access to laboratory services (including fee-for-service agreements)? ***Pull down menu with options: Yes; No***

g. If yes, briefly describe this agreement and the laboratory services that are obtained.

Field to enter free text.

Category 3: Responsible persons

16. Complete the following table to identify the responsible person(s) to oversee implementation, performance, and enforcement of the specified activities and measures:

Role	Name	Title	Phone Number(s)	Email
Animal Health Activities and Measures				
Responsible person	<i>Free text</i>	<i>Free text</i>	<i>Numbers formatted as (XXX)-XXX-XXXX</i>	<i>Free Text;</i>
Wildlife Activities and Measures				
Responsible person				

Category 4: Program animal demographics

17. Complete the following table to describe the cattle, bison, and captive cervid herds in the State or Tribe.

Brucellosis and Bovine Tuberculosis Program Standards

	Number of animals	Number of herds	Type	Source of Estimate
Cattle				
Cattle	<u>Number</u>	<u>Number</u>	Dairy	<u>Pull down:</u> NASS State census or survey State animal health records Estimated
Cattle			Beef	
Cattle			Mixed	
Bison				
Domestic Bison				
Captive Cervids				
Captive Cervid			Breeding	
Captive Cervid			Shooter/Hunting	
Captive Cervid			Other	

18. (Optional Question) Briefly describe the geographic distribution of these herds of program animals within your State or Tribe. A map illustrating the distribution may also be attached.

Field to enter free text.

Ability to attach PDF or include URL of coverage map

19. Complete the following table with the number of animal concentration points within your State or Tribe.

Concentration Point	Number of Concentration Points	Source of Estimate
Livestock Markets	<u>Number</u>	<u>Pull down:</u> NASS State census or survey State animal health records Estimated
Sale Barns		
Buying Stations		
Feedyards		
Slaughtering Establishments		

Ability for user to add rows to table as needed

20. Briefly describe the geographic distribution of these concentration points within your State or tribe. A map illustrating the distribution may also be attached.

Field to enter free text.

Ability to attach PDF or include URL of coverage map

21. (Optional Question) Briefly describe the general movement patterns of program animals within your State or tribe. A map illustrating the patterns may also be attached.

Field to enter free text.

Ability to attach PDF or include URL of coverage map

Category 5: Surveillance

A. National Surveillance

22. Confirm that your State or Tribe agrees to participate in the National Surveillance Plan for Brucellosis.

Pull down menu with options:

Yes, we agree to participate.

No, we are submitting an alternate plan for APHIS approval.

- h. If no, please attach a document describing the alternate plan that is proposed.

Ability to attach PDF

23. Confirm that your State or Tribe agrees to participate in the National Surveillance Plan for Bovine Tuberculosis.

Pull down menu with options:

Yes, we agree to participate.

No, we are submitting an alternate plan for APHIS approval.

- i. If no, please attach a document describing the alternate plan that is proposed.

Ability to attach PDF

24. Describe the core and refresher training provided to BTB QAVs in your State or Tribe including the elements covered in the training and the approach used to verify that trainees can demonstrate specific skills. A document outlining the training may also be attached.

Field to enter free text. Ability to attach PDF

25. Describe how performance of BTB QAVs is monitored in your State or Tribe including the actions taken when a BTB QAV fails to meet the performance standards. A document outlining the process may also be attached.

Field to enter free text. Ability to attach PDF

Targeted Surveillance in Source Populations

26. Describe your approach to conducting the required surveillance in any source populations of wildlife that pose a risk of transmission to program animals in your State or Tribe (as identified in Category 6, Question 28). Include information about how the sample size was determined and the process for sample selection/collection in your description.

Field to enter free text. Ability to attach file.

B. Targeted Surveillance in At-Risk Populations

27. Describe your approach for conducting the required annual testing of herds in any at-risk population of program animals in your State or Tribe (as identified in Category 6, Question 29). Include a goal for detection level, sample size goal, and sample selection process in your description.

Field to enter free text. Ability to attach file.

Category 6: Sources of brucellosis and bovine tuberculosis

28. List known sources of brucellosis and/or bovine tuberculosis that pose a risk of disease introduction to program animals within your State or Tribe.

Disease of Concern	Source Population Type/Name	Approximate Population Size		Geographic Distribution of Population	Approximate Prevalence	Other Factors that Contribute to Risk of Disease Spread
		Number of Herds/Wildlife Populations	Number of Animals			
<i>Pull down: Brucellosis TB No known source for either Brucellosis or TB</i>	<i>Free text</i>	<i>Number</i>	<i>Number</i>	<i>Free text</i>	<i>Number</i>	<i>Free text;</i>

Ability for user to add rows to table as needed.

29. For each source population listed, summarize the likelihood of transmission of the disease of concern to program animals within your State or Tribe. Include a description of:

- a. The potential for exposure of program animals to these source populations.
- b. Factors that may influence possible exposure (i.e., the movement patterns of animals, seasonal variations in exposures, etc.). {NOTE: Do not list mitigations that your State or Tribe would implement here. These will be described in Category 7.}
- c. If available, cite or attach a summary of key findings from any formal risk assessments concerning the risk of spread of brucellosis or bovine tuberculosis.

Field to enter free text. Ability to attach PDF

30. If no source populations were listed for either disease, provide a justification for this conclusion. Include a description of the information considered and the process used to reach this conclusion. If available, cite or attach a summary of any formal risk assessments concerning the risk of spread of brucellosis or bovine tuberculosis.

Field to enter free text. Ability to attach PDF

Category 7: Risk mitigation measures

31. If sources of brucellosis or bovine tuberculosis that pose a risk of disease introduction into program animals were identified (Questions 28 and 29), describe the measures that your State or Tribe has implemented or will implement to mitigate this risk.

Field to enter free text.

Category 8: Disease investigation, management, and response

32. Confirm that your State or Tribe agrees to meet the minimum requirements for epidemiological investigations and affected herd management as set forth in 9 CFR 76.7.

Pull down menu with options:

Yes, we agree to meet the minimum requirements.

No, we are submitting an alternate plan for APHIS approval.

- j. If no, attach a document describing any epidemiological investigation and/or affected herd management activities that your State or Tribe plans to take in response to occurrences of disease that differ from or exceed these requirements.

Ability to attach PDF

Public Notification

33. Confirm that your State or Tribe is aware that the animal health plan and any future amendments to the plan will be publicly available (confidential information will be redacted).

Radio button next to the following: Yes, we are aware that the plan and future amendments will be publicly available.

34. Confirm that your State or Tribe is aware that required reports will be publicly available (confidential information will be redacted).

Radio button next to the following: Yes we are are aware that required reports will be publicly available.

Brucellosis and Bovine Tuberculosis Program Standards

Electronic Signatures:

Designated Brucellosis Epidemiologist

Designated Tuberculosis Epidemiologist

AD

State or Tribal Responsible Person – Animal Health Activities

State or Tribal Responsible Person – Wildlife Activities

Appendix 2: Statuses Applied to States and Tribes

To date, no statuses have been assigned to States or Tribes.

Appendix 3: Annual Report Template

Annual Reporting for State, Tribe, State’s Recognized Management Area (RMA), or Tribe’s RMA (Include RMA designation/identifier if more than one RMA in a State or tribe)

Program Animal Demographics

<input type="checkbox"/>	<input type="checkbox"/>	Cattle		Privately owned Bison		Captive Elk		Other cervid species*	
Check Box if number Source is NASS	Check Box if numbers come from another source and indicate source below								
Select Area described in Report		Total	Herds	Total	Herds	Total	Herds	Total	Herds
Choose an item.									

* List captive cervid species separately by species (add columns or separate table, if necessary)

Brucellosis

Accredited Brucellosis Herds

	Cattle			Privately Owned Bison	Elk*	**Other cervid species	Other cervid species	Other cervid species	Other cervid species
	Beef	Dairy	Mixed						
Number of accredited Brucellosis herds									

* Captive elk

** List captive cervid species separately by species (add columns or separate table, if necessary)

Brucellosis and Bovine Tuberculosis Program Standards

National Brucellosis Slaughter Surveillance **

Program Animals	Number of program animals slaughtered	Number of program animals brucellosis sampled	Brucellosis test interpretation of brucellosis samples collected			Number of brucellosis affected herds detected	Comments
			N*	S*	R*		
Cattle							
Privately Owned Bison							

* N = negative S = suspect R = Reactor

** Indicate NA in the comments section if no slaughter facilities in the State, Tribe, or RMA have been designated for national brucellosis slaughter surveillance sample collection

Brucellosis and Bovine Tuberculosis Program Standards

State Brucellosis Slaughter Surveillance (Slaughter sampling not included in the National Brucellosis Surveillance Plan)

Program Animals	# Tested at Inspected Slaughter Facilities	# Tested at Custom Kill Slaughter Facilities	Brucellosis test interpretation of brucellosis samples collected			Number of brucellosis affected herds detected	Comments
			N*	S*	R*		
Cattle							
Privately Owned Bison							
Elk							
Whitetail deer							
Mule deer							
Fallow deer							
Reindeer							
**							

* N = negative S = suspect R = Reactor

** Add rows or create a separate table for each additional captive cervid species

Brucellosis and Bovine Tuberculosis Program Standards

Wildlife Surveillance Testing

Species of Wildlife	# Tested by Wildlife Officials	#Tested by Hunter Harvest	# Tested at State Inspected Slaughter Facilities	#Tested at Custom Kill Slaughter Facilities	Brucellosis test interpretation of brucellosis samples collected		
					N*	S*	R*
**							

* N = negative S = suspect R = Reactor

** Add rows or create a separate table for each additional cervid species

Brucellosis and Bovine Tuberculosis Program Standards

State Active Brucellosis Surveillance

Reason for testing	Species*	# Whole herd tests	# Herds	# Head	# Negative	# Suspect	# Reactor
Area testing							
Milk Ordinance testing							
Movement testing – Intrastate							
Movement testing – Interstate							
Change of ownership testing (Outside of First Point Testing)							
Epidemiological investigation testing (includes diagnostic testing)							
First point testing							
Herd accreditation testing							
International Import/export testing							
Exhibition & show testing							

* Add additional rows or tables if more than one program species was tested for one or more of the reasons for testing.

Program Animals Officially Brucellosis Vaccinated

	Number
Program cattle brucellosis officially calfhood vaccinated in this reporting period	
Number of program bison officially brucellosis calfhood vaccinated in this reporting period	
Number of program cattle officially brucellosis adult vaccinated in this reporting period	
Number of program bison officially brucellosis adult vaccinated this reporting period	

Brucellosis Narratives

1. Describe how all slaughter (includes national surveillance plan, state inspected, and custom kill) facility and on-farm reactors and suspects were identified, epidemiological investigations are being or were performed, and reasons affected herd cases and trace epidemiological investigations were closed. Discuss reactors and suspects in separate sections. In the reactor section, provide the most detail on investigating and closing of reactor cases, including affected herd cases. In the suspect section, discuss the epidemiological investigation and evaluation of suspects and epidemiological traces in general terms and/or as a summary of all suspects.
2. Specifically discuss the number, if any, of untraceable animals, the reasons for traceability problems, and possible solutions to reduce the number of untraceable animals and correct traceability problems.
3. For States with slaughter plants that have been designated for national brucellosis surveillance sampling, discuss problems with sampling appropriate animals, sample collection, animal identification, and traceability at and from the slaughter plant(s) in your state and efforts being made to correct the problems and recommended solutions.

In addition if this report is for an RMA

1. Surveillance
Discuss any problems with identification and traceability at markets, collection points, and at change of ownership testing within and outside of the RMA and any proposed solutions.
2. Herd Plans
 - a. Discuss general characteristics of any individual herd plans for herds in the RMA and herds that temporarily reside inside the RMA.
 - b. Evaluate any problems with the herd testing schedules.

Bovine Tuberculosis (bTB)

Accredited Bovine Tuberculosis Herds

	Cattle			Privately Owned Bison	Elk*	White tail deer	**Other cervid species	Other cervid species	Other cervid species
	Beef	Dairy	Mixed						
Number of accredited bovine TB herds									

* Captive elk

** List each captive cervid species separately by species (add columns or additional tables, if necessary)

National Bovine Tuberculosis Surveillance (State Inspected Plants)

Facility ID	Number of inspection visits this reporting period	Number of Samples Submitted from State Inspected Slaughter Facilities	Number of Samples Submitted from Custom Kill Slaughter Facilities	# of Bovine Samples Submitted	# of Cervid Samples Submitted	Number Negative	Number Confirmed Positive	# of bTB affected herds detected

* Add rows or create a separate table for each additional cervid species

Bovine Tuberculosis Caudal Fold Tuberculin Testing Standard

Number of TB Qualified Accredited Veterinarians (BTB QAV) in State, Tribe, or RMA	
Number of BTB QAV conducting any CFT testing during the reporting period:	
Number of BTB QAV accumulating ≥ 300 CFT tests performed during the reporting period:	
Number of BTB QAV accumulating ≥ 300 CFT tests performed during the reporting period, that met or exceeded the national performance standard:	
Percentage of BTB QAV accumulating ≥ 300 CFT tests performed during the reporting period, that met or exceeded the national performance standard:	

Bovine Tuberculosis Whole and Partial Herd Testing

Reason for testing	Species*	# Whole Herd Tests	# Herds	# Head	# Negative	# Positive	# Necropsied	# Lesions collected
Area Testing								
Milk Ordinance testing								
Movement testing – Intrastate								
Movement testing - Interstate								
Change of ownership testing								
Epidemiological investigation testing (includes diagnostic testing)								
Herd accreditation testing								
International Import/export testing								
Exhibition & show testing								

* Add addition rows if more than one program species was tested for one or more of the reasons for testing.

Bovine Tuberculosis Narratives

1. Describe how all confirmed *M. bovis* slaughter cases (includes national surveillance plan, State-inspected, and custom kill) were identified, how epidemiological investigations are being or were performed, and the reasons affected herd cases and trace epidemiological investigations were closed.

Brucellosis and Bovine Tuberculosis Program Standards

2. Discuss animals classified as Reactors to bTB tests. Provide necropsy, histopathological, PCR and culture results for each reactor case. Describe the epidemiological investigations and closing of *M. bovis* positive cases, including affected herd cases. A copy of the current situation report may be attached.
3. Specifically discuss the number of untraceable animals, if any, the reasons for traceability problems, and possible solutions to reduce the number of untraceable animals and correct traceability problems.
4. Discuss problems with sampling appropriate animals, sample collection, animal identification, and traceability at and from the slaughter plant(s) in your State, Tribe, or RMA and efforts being made to correct the problems and recommend solutions.
5. Provide a narrative describing the actions taken when Qualified Accredited Veterinarians do not meet the national standard.

In addition if the report is discussing a RMA:

1. Herd Plans
 - a. Discuss general characteristics of the individual herd plans for herds in the RMA.
 - b. Evaluate the herd testing schedules and reasons, if any, for less than annual testing.

See Annual Review of Animal Health Plan below.

Annual Review of Animal Health Plan

Review each category of your State or Tribe's approved animal health plan and confirm that the plan is still accurate and current.*

Animal Health Plan Category	I confirm the plan is accurate and current.	A change in the plan has occurred for this category and information in the plan needs to be updated for this category.
Category 1: Laws and Regulations	<i>Check box</i>	<i>Check box</i>
Category 2: Organization and Infrastructure	<i>Check box</i>	<i>Check box</i> <i>If checked, would bring up Category 3 from AHP.</i>
Category 3: Responsible persons	<i>Check box</i>	<i>Check box</i> <i>If checked, would bring up Category 4 from AHP.</i>

Brucellosis and Bovine Tuberculosis Program Standards

Category 4: Program animal demographics	<i>Check box</i>	<i>Check box</i>
Category 5: Surveillance	<i>Check box</i>	<i>Check box</i>
Category 6: Sources of brucellosis and bovine tuberculosis	<i>Check box</i>	<i>Check box</i>
Category 7: Risk mitigation measures	<i>Check box</i>	<i>Check box</i>
Category 8: Disease investigation, management, and response		
Recognized Management Area (If applicable)	<i>Check box</i>	<i>Check box</i>

*Amendments may be attached to the annual report. However, for substantive changes to the AHP, please refer to § 76.2 and Element 1 of these standards; an amended AHP may need to be submitted sooner than the annual report.

Brucellosis and Bovine Tuberculosis Program Standards

Name	Signature	Date
State or Tribal Responsible Person – Animal Health Activities		
State or Tribal Responsible Person – Wildlife Activities		
Associate Director		

Amendment to Animal Health Plan for Recognition of a Management Area (RMA)

State/Tribe: *Select State from a pull down listing all States and select Tribes.*

Disease of Concern: *Select from a pull down listing Brucellosis and TB*

Brucellosis and Bovine Tuberculosis

Annual Report Template Instructions

Identify the State, Tribe, or recognized management area (RMA) for which the annual report is being submitted by clicking on the “Choose an item” button and select the appropriate designation. If the State or Tribe has more than one RMA, identify each RMA using a distinct name, number, etc. on the annual report submitted. A separate RMA annual report must be submitted for each RMA in a State or Tribe.

Program Animal Demographics

Purpose: To provide statistical and epidemiological data for disease program management and national reports. This section provides a summary of program animal demographics within the State, Tribe, or RMA.

Indicate by checking the appropriate box if the data was sourced from NASS or another source and indicate what that source is.

Provide the total number of individual program animals and the total number of program animal herds by species.

List each captive elk herd separately from other captive cervids species. Add additional columns or a separate table if more than two cervid species are being reported.

Brucellosis

Accredited Brucellosis Herds

Purpose: To provide statistical and epidemiological data for disease program management and national reports. This section provides a summary of program animal brucellosis accredited herds within the State or Tribe.

Provide the total number of brucellosis accredited herds in the State or Tribe.

For brucellosis accredited cattle herds, provide the data for each type of cattle herd, i.e. beef, dairy, and mixed.

List each captive cervid species separately. Add additional columns or a separate table if more than five captive cervid species are being reported.

National Brucellosis Slaughter Surveillance

Purpose: To provide statistical brucellosis surveillance data for disease program management, national brucellosis reports, and verification of the country’s disease status. This section provides a summary of program animals slaughtered within and from the State, Tribe, or RMA as part of the National Brucellosis Slaughter Surveillance plan.

Provide the total number of program animals slaughtered, by species, at slaughter facilities participating in the national slaughter surveillance plan within and from the State, Tribe, or RMA.

Brucellosis and Bovine Tuberculosis Program Standards

Provide the total number of program animals, by species, from the State, Tribe, or RMA that were brucellosis sampled at slaughter facilities participating in the national slaughter surveillance plan.

Provide total number of negative, suspect, and reactor brucellosis test classifications for program animals, by species, sampled at slaughter facilities participating in the national slaughter surveillance plan.

Provide the total number of brucellosis affected herds, by species, detected by brucellosis sampling at slaughter facilities participating in the national slaughter surveillance plan.

As an attachment: provide comments, if any, regarding national brucellosis slaughter surveillance.

Brucellosis Slaughter Statistics (Slaughter Surveillance not included in the National Surveillance Plan)

Purpose: To provide statistical brucellosis surveillance data for disease program management, national brucellosis reports, and verification of the country's disease status. This section provides a summary of program animals slaughtered within the State, Tribe, or RMA that were not part of the National Brucellosis Slaughter Surveillance plan.

Provide the total number of program animals, by species, brucellosis sampled at State-inspected slaughter facilities.

Provide the total number of program animals, by species, brucellosis sampled at State-inspected custom kill slaughter facilities.

Provide the total number of negative, suspect and reactor brucellosis test classifications for program animals, by species, sampled at State inspected slaughter and custom kill slaughter facilities.

Provide the total number of brucellosis affected herds, by species, detected at State inspected slaughter and custom kill facilities.

As an attachment: provide comments, if any, regarding brucellosis surveillance at state inspected slaughter and custom kill slaughter facilities.

Wildlife Surveillance Testing

Purpose: To provide statistical brucellosis surveillance data for disease program management, national brucellosis reports, and verification of the country's disease status. This section provides a summary of wildlife brucellosis surveillance within the State, Tribe, or RMA.

Indicate each species of wildlife tested.

Provide the number of each wildlife species tested by wildlife officials.

Provide the number of each wildlife species tested by hunter harvest testing.

Provide the number of each wildlife species tested at State inspected slaughter facilities.

Brucellosis and Bovine Tuberculosis Program Standards

Provide the number of each wildlife species tested at State custom kill slaughter facilities.

Indicate the numbers of each test interpretation for the brucellosis samples collected.

State Active Brucellosis Surveillance

Purpose: To provide statistical brucellosis active surveillance data for disease program management, national brucellosis reports, and verification of the country's disease status. This section provides a summary of program animals officially brucellosis whole and partial herd testing as part of active brucellosis surveillance within the State, Tribe, or RMA.

Reason for testing – the left column lists the reasons for in-State testing.

Identify the species for each reason for testing listed. Add a separate row or table for each species if more than one program animal species was tested for one or more testing reasons listed.

Test data from whole herd and partial herd testing for each reason for brucellosis testing may be combined, i.e. total number of animals tested, herds tested, and brucellosis test classifications.

Provide the number of negative, suspect, and reactor brucellosis test classification for each of the testing reasons listed.

Program Animals Officially Brucellosis Vaccinated

Purpose: To provide statistical and epidemiological data for disease program management and national reports. This section provides a summary of program animals officially brucellosis vaccinated within the State, Tribe, or RMA.

Provide the total number of cattle and privately owned bison that were brucellosis officially calfhood vaccinated in the State, Tribe, or RMA during the reporting period.

Provide the total number of cattle and privately owned bison, by species, that were brucellosis officially adult vaccinated in the State, Tribe, or RMA during the reporting period.

Brucellosis Narratives

Purpose: To provide information regarding how the State or Tribe managed specific issues, including but not limited to: Problems with epidemiological traces; lack of cooperation with surveillance sampling; and any issues with control and eradication of brucellosis that are not addressed or insufficiently addressed in other areas of the annual report.

Describe how all slaughter (includes national surveillance plan, State inspected slaughter facility, custom kill) facility brucellosis reactors and suspects were identified, how epidemiological investigations are being or were performed, and the reasons affected herd cases and trace epidemiological investigations were closed. Discuss brucellosis test classification reactors and suspects in separate sections.

Brucellosis and Bovine Tuberculosis Program Standards

1. Brucellosis reactor section – Describe, in detail, the epidemiological investigation of animals classified as reactors, including but not limited to:
 - a. How and when the brucellosis reactor or reactors were detected and when a case situation report was initiated;
 - b. How the brucellosis reactors were traced to their herd of origin;
 - c. Source herd brucellosis testing performed and scheduled;
 - d. Brucellosis classification of the source herd, i.e. affected or not affected;

If the source herd was classified as an affected herd, describe the actions taken and proposed regarding management of the affected herd. A copy of the brucellosis affected herd plan and an up to date brucellosis case situation report may be submitted to fulfill this requirement.

- e. If brucellosis source herd case was closed, provide justifications for closing the case.
- f. Brucellosis epidemiological investigations of herds from which animals were traced into the source herd and disposition of those trace-in herds;

If trace-in epidemiological investigations were closed, provide justifications for closing each trace-in investigation.

- g. Brucellosis epidemiological investigations of herds to which animals were traced out of the source herd and disposition of those trace-out herds.

If trace-out epidemiological investigations were closed, provide justifications for closing each trace-out investigation.

2. Brucellosis suspect section: Describe in general terms the epidemiological investigation of animals classified as suspects, including but not limited to, evaluation criteria used to classify animals as suspects.
3. Discuss the number of brucellosis test classification reactor and suspect animals that were untraceable, including but not limited to:
 - a. The reasons the animals could not be traced after they were classified as a suspect or reactor;
 - b. The reasons trace-in or trace-out animals from an affected herd could not be traced;
 - c. Provide possible solutions and to reduce the number of untraceable animals and correct traceability problems.
4. For States with slaughter facilities that have been designated for national brucellosis surveillance sampling, discuss;
 - a. Problems, if any, with sampling appropriate animals;
 - b. Sample collection problems, if any, at the slaughter facility;
 - c. Animal identification collection problems, if any, at the slaughter facility;

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- d. Traceability problems, if any, of animals at and from the slaughter facilities;
 - e. Efforts being made to correct the problems at the slaughter facility; and
 - f. Recommend possible solutions to correct the problems.
5. Discuss the following regarding brucellosis surveillance sampling at State inspected slaughter and custom kill facilities;
- a. Problems, if any, with sampling appropriate animals;
 - b. Sample collection problems, if any, at the slaughter facility;
 - c. Animal identification collection problems, if any, at the slaughter facility;
 - d. Traceability problems, if any, of animals at and from the slaughter facilities;
 - e. Efforts being made to correct the problems at the slaughter facility; and
 - f. Recommend possible solutions to correct the problems.

In addition, if the annual report is discussing a RMA:

1. Discuss animal identification problems, if any, at markets, collection points, and at change of ownership testing within the RMA.

Discuss proposed and/or implemented solutions to correct the animal identification problems at markets, collection point, and at change of ownership testing within the RMA.

2. Discuss animal identification problems, if any, at markets and collection points outside of the RMA.

Discuss proposed and/or implemented solutions to correct the animal identification problems at markets and collection point outside of the RMA.

3. Herd Plans

Discuss the general characteristics of the individual herd plans for unaffected herds and herds that temporarily reside in the RMA, including but not limited to:

- a. The criteria used to determine herd testing schedules. If the herds are not tested annually, provide the reasons for not testing the herds annually; and
- b. Brucellosis transmission mitigation measures or actions.

4. **If there have been changes to the boundaries, methods, or management in the RMA, the Animal Health Plan must be amended**

Bovine Tuberculosis (bTB)

Accredited bTB herds

Purpose: To provide statistical and epidemiological data for disease program management and national reports. This section provides a summary of program animal bTB accredited herds within the State or Tribe.

Provide the total number of bTB accredited herds in the State or Tribe.

For bTB accredited cattle herds, provide the data for each type of cattle herd, i.e. beef, dairy, and mixed.

List each captive cervid species separately. Add additional columns or a separate table if more than five captive cervid species are being reported.

National Bovine Tuberculosis Surveillance (State Inspected Plants)

Purpose: To provide statistical bTB surveillance data for disease program management, national bTB reports, and verification of the country's disease status. This section provides a summary of program animals slaughtered within the State, Tribe, or RMA and were sampled for bTB.

Provide the facility ID number of the plant inspected.

Provide the total number of inspection visits conducted at the State inspected and custom kill slaughter facilities.

Provide the total number of program animals bTB sampled at State inspected slaughter facilities.

Provide the total number of program animals bTB sampled at State inspected custom kill slaughter facilities.

Provide the species-by-species breakdown of this sampling.

Provide total number of negative and positive bTB test classifications for program animals, by species, sampled at State inspected slaughter and custom kill facilities.

Provide the total number of bTB affected herds, by species, detected at State inspected slaughter and custom kill facilities.

As an attachment: provide comments, if any, regarding brucellosis surveillance at State inspected slaughter and custom kill facilities.

Bovine Tuberculosis Caudal Fold Tuberculin Testing Standard

Purpose: To provide a report of summary information about the percentage of qualified accredited veterinarians that meet or exceed the performance standard to VS annually. The competency of accredited veterinarians to classify skin test reactions correctly is central to the control and eradication of bTB and is evaluated based on a minimum performance standard for the test.

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Provide the number of qualified accredited veterinarians (BTB QAV) in the State, Tribe, or RMA.

(BTB QAV = Federally Accredited Veterinarians qualified by the bTB program to perform official bTB testing.)

Provide the number of BTB QAVs conducting any caudal fold testing during the reporting period.

Provide the number of BTB QAVs accumulating ≥ 300 caudal fold tests performed during the reporting period.

Provide the number of BTB QAVs accumulating ≥ 300 caudal fold tests performed during the reporting period, that met or exceeded the national performance standard.

Provide the percentage of BTB QAVs accumulating ≥ 300 CFT tests performed during the reporting period, that met or exceeded the national performance standard.

Divide the number of BTB QAVs accumulating ≥ 300 CFT tests performed during the reporting period that met or exceeded the national performance standard by the number of BTB QAVs accumulating ≥ 300 CFT tests performed during the reporting period.

Bovine Tuberculosis Whole and Partial Herd Testing

Purpose: To provide statistical bTB active surveillance data for disease program management, national bTB reports, and verification of the country's disease status. This section provides a summary of program animals officially bTB whole and partial herd testing as part of active bTB surveillance within the State, Tribe, or RMA.

Indicate reason for testing.

Identify the species for each testing reason listed. Add a separate row or table for each species if more than one program animal species was bTB tested for one or more of the testing reasons listed.

Whole herd testing – all bTB test eligible animals in a herd were tested.

Herd (partial) testing – a portion bTB test eligible animals in a herd were tested.

Provide the number of negative, suspect and reactor bTB test classification for each testing reason listed. Test data from whole herd and partial herd testing for each reason for bTB testing may be combined, i.e. total number of animals tested, herds tested and bTB test classifications.

Provide the numbers necropsied and number of lesions collected and submitted to the lab.

bTB Narratives

Purpose: To provide information regarding how the State or Tribe managed specific issues, including but not limited to: problems with epidemiological traces; lack of cooperation with surveillance sampling; and any issues with control and eradication of bTB that are not addressed or insufficiently addressed in other areas of the

Brucellosis and Bovine Tuberculosis Program Standards

annual report. Provide affected herd information for each brucellosis affected herd detected in the State, Tribe, or RMA during the reporting period.

Describe how all slaughter (includes national surveillance plan, State inspected slaughter facility, custom kill) facility reactors and suspects were identified, how epidemiological investigations are being or were performed, and the reasons affected herd cases and trace epidemiological investigations were closed. Discuss bTB test classification positives and suspects in separate sections.

1. bTB reactor section – Describe, in detail, the epidemiological investigation of animals classified as bTB positives, including but not limited to:
 - a. How and when the bTB positive or positives were detected and when a case situation report was initiated;
 - b. How the bTB positives were traced to their herd of origin;
 - c. Source herd bTB testing performed and scheduled;
 - d. bTB classification of the source herd, i.e. affected or not affected;
If the source herd was classified as an affected herd, describe the actions taken and proposed regarding management of the affected herd. A copy of the bTB affected herd plan and an up to date bTB case situation report may be submitted to fulfill this requirement.
 - e. If bTB source herd case was closed, provide justifications for closing the case.
 - f. bTB epidemiological investigations of herds from which animals were traced into the source herd and disposition of those trace-in herds;
If trace-in epidemiological investigations were closed, provide justifications for closing each trace-in investigation.
 - g. bTB epidemiological investigations of herds to which animals were traced out of the source herd and disposition of those trace-out herds.
If trace-out epidemiological investigations were closed, provide justifications for closing each trace-out investigation.
2. bTB suspect section: Describe in general terms the epidemiological investigation of animals classified as bTB suspects, including but not limited to, evaluation criteria used to classify animals as suspects.
3. Discuss the number of bTB test classification positive and suspect animals that were untraceable, including but not limited to:
 - a. The reasons the animals could not be traced after they were classified as a suspect or positive;
 - b. The reasons trace-in or trace-out animals from an affected herd could not be traced;
 - c. Provide possible solutions and to reduce the number of untraceable animals and correct traceability problems.
4. Discuss the following regarding bTB surveillance sampling;
 - a. Discuss problems, if any, with sampling appropriate animals;
 - b. Sample collection problems, if any, at the slaughter facility;

Brucellosis and Bovine Tuberculosis Program Standards

- c. Animal identification collection problems, if any, at the slaughter facility;
 - d. Traceability problems, if any, of animals at and from the slaughter facilities; and
 - e. Efforts being made to correct the problems at the slaughter facility.
5. Provide a narrative describing the actions taken when Qualified Accredited Veterinarians do not meet the national standard.

In addition, if the annual report is discussing a RMA:

Herd Plans

Discuss the general characteristics of the individual herd plans for unaffected herds and herds that temporarily reside in the RMA, including but not limited to:

- a. The criteria used to determine herd testing schedules. If the herds are not tested annually, provide the reasons for not testing the herds annually; and
- b. bTB transmission mitigation measure or actions.

If there have been changes to the boundaries, methods, or management in the RMA, the Animal Health Plan must be amended.

Annual Review of Animal Health Plan

Review each category of your State or Tribe's approved animal health plan and confirm that the plan is still accurate and current.

Click the appropriate check box and appropriate answer selection.

If there has been a change in Category 3 and/or 4, provide the appropriate information.

Provide the printed names, signatures and date signed for the following:

State or tribal responsible person – Animal Health Activities

State or tribal responsible person – Wildlife Activities

Associate Director

If an amendment to the State or Tribe Animal Health Plan for recognition of a management area (RMA) is being submitted, select the appropriate state or tribe from the pull down listing of all States and tribes. Select the Disease of Concern from the pull down listing, i.e. brucellosis or bovine tuberculosis.

Submission of the Annual Report

While APHIS prefers States and Tribes to submit annual reports electronically, they can submit a paper copy to the Associate Director ([Link to Area Office list](#)).

Appendix 4 A: Animal Trace-in Documentation: Brucellosis

Form Approved OMB NO. 40-R4028

See Notice/Instructions on Reverse
U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
VETERINARY SERVICES

ORIGIN OF REACTORS/HERD ADDITIONS		INSTRUCTIONS: If animals are traceable to a herd source, obtain all supporting documents (i.e., health certificates, sales yard releases, brand inspection records, test charts, sales slips, etc.)			1. NAME OF HERD OWNER		2. DOCUMENT NUMBER						
SUPPLEMENTAL TO VS FORM 4-108					3. COUNTY		4. HERD NO.						
					PAGE		OF PAGES						
SOURCE NAME AND ADDRESS (Include all reactors both raised & purchased) A	Date Added B	Most Recent Status of Animals (N, H, or "N") C	IDENTIFICATION Tag Nos. D		Brand E	Sex F	Age when purchased G	Breed H	Vac. Status I	Specimen C Submitted J	Results K	REMARKS Date of last culling (Give if culling Disposition of calf (if in herd) L	STATUS AT ENTRY HERD M
<p>7. Have arrangements been made to contact owners of source herds of all purchased suspects and reactors? <input type="checkbox"/> Yes <input type="checkbox"/> No. (If no, explain on reverse)</p> <p>VS FORM 4-108A (OCT 80) <i>Previous editions obsolete</i></p>													

Brucellosis and Bovine Tuberculosis Program Standards

NOTICE TO HERD OWNERS

Herd Owners (*Managers or Agents*) of herds of brucellosis affected livestock must be advised that information is given voluntarily to assist in the elimination of brucellosis from the livestock population. Cooperation of all affected herd owners (*Managers or Agents*) is needed to complete a thorough epidemiologic investigation to identify the source of the disease, the method of spread and the possible dissemination to new herds. The authorities under which the brucellosis program is conducted are contained in 21 USC 111, 112, 114, 114a, 114a-1, 115, 120, 121, 125, and 134a-f and Title 9, Code of Federal Regulations, Part 51 and Part 78.

INSTRUCTIONS

The purpose of this form is to record information of epidemiologic importance individually for (1) each MCI reactor (*if any*), (2) each reactor on the initial herd test, and (3) by lot for any other purchases. In most cases, this should cover the 2-year period prior to discovery of the infection but the time period may vary on epidemiologic findings.

- 1-4. From 4-108. Item 2 is the preprinted number on the 4-108.
5. Indicate which page of 4-108A this is and how many total pages were completed.
- 6-A. Record the complete legal name and mailing address of the supplier for each animal or group of animals added to herd. List each MCI reactor (*if any*) on a separate line. List each farm reactor on the initial herd test on a separate line. List each purchase (*one purchase lot per line*) for all other animals added to the herd over the past 2 years (*or longer at the discretion of the investigator*).
- 6-B. List the date the present owner acquired control of the animal(s).
- 6-C. Indicate the latest status of the animal(s) by "M" (*MCI reactor*), "R" (*herd reactor*), or "N" (*negative*).
- 6-D. List the identification other than brand for each reactor animal when purchased if known and when found to be a reactor and the reactor tag number. For purchase lots indicate number of animals involved and list the tag range (*if in series*), state codes, or other available identification.
- 6-E. List all brands on the animal(s) or listed on any permit received with the animals.
- 6-F. Indicate "F" for female and "M" for male. For purchase lot data record the number of each.
- 6-G. Indicate the age when purchased. For purchase lots record the age range and the average or most frequent age.
- 6-H. Record the breed of reactors if known. If nondescript, record the most probable breed by appearance, etc. For purchase lots record the predominant breed or as mixed beef or mixed dairy.
- 6-I. Record the brucella vaccination status as "CV" (*calfhood*), "AV" (*adult*), "NV" (*not vaccinated*), or "UN" (*unknown*).
- 6-J. Indicate material submitted for brucella culture. Use "T" for lymphoid and other glandular tissue. Use "F" for fetus, placenta or uterine exudates. Use "M" for milk including any udder secretion. If two or more were submitted, list one above the other.
- 6-K. Record brucella culture results as "N" (*negative*), "1" (*type 1*), "2" (*type 2*), "4" (*type 4*), "19 S" (*Strain 19*), "S" (*suis*), and "U" (*contaminated or unsatisfactory*). If milk, placentas and/or tissues are submitted, record the results for each material.
- 6-L. For reactors record the date of last calving (*circle the date if it was an abortion or a full term dead or weak calf*), indicate where the calf is now, and any other information considered pertinent. For purchase lots record pertinent information such as culled aborters, etc.
- 6-M. Enter the respective code number(s); 1-untested, 2-negative test, 3-from a brucellosis certified free herd, 4-from a certified free (*or class A*) area.
7. Indicate "yes" if you are going to contact all herds of origin of reactors or other suspicious purchases. Indicate "no" if you are not going to contact all and explain on back of form.
8. Signature of Veterinarian responsible for the completion of this investigation.
9. Date signed by Veterinarian responsible for this investigation.

Attach copies of any supporting documents that might help trace additions. This could be market invoices and release slips, brand inspections, health certificates, receipts, etc.

Appendix 4 B: Animal Trace-in Documentation: Tuberculosis

VS Form 6-4A

Print Form

U. S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
VETERINARY SERVICES

INVESTIGATION FOR EVIDENCE OF TUBERCULOSIS (Reactor)

CODES - (The following codes are to be used under the respective column headings)

REASON FOR TEST (20)	HERD TEST LESION CODE (21)	SEX (34)	REACTOR LESION (35)	LAB RESULTS (36)
1. AREA	DENOTES ALL LOCATIONS OF LESIONS FOUND IN ALL OF THE REACTORS ON A HERD TEST	1. FEMALE	0. NO P.M. RPT.	1. COMPATIBLE
2. HERD ACCREDITATION OR REACCREDITATION		2. MALE	1. NGL	2. SUGGESTIVE
3. COMPLY WITH MILK ORDINANCES	BREED (33)	3. STEER	2. SKIN	3. NOT SUGGESTIVE
4. SALE, SHOW, INTERSTATE, INTRA-STATE OR EXPORT SHIPMENT		1. HOLSTEIN	4. THORACIC	3. HEAD
5. IMPORTED ANIMALS (interstate or international)	6. AYRSHIRE	5. ABDOMINAL	4. THORACIC & ABDOMINAL	
6. RETEST OF QUARANTINED HERD	7. SHORTHORN	6. HEAD & THORACIC	5. THORACIC & ABDOMINAL	
7. AFTER TRACING ANIMALS WITH LESIONS REGULAR KILL	8. SWISS	7. HEAD & THORACIC	6. HEAD & THORACIC & ABDOMINAL	
8. AFTER TRACING REACTING ANIMALS	9. OTHER	8. THORACIC & ABDOMINAL	7. HEAD & THORACIC & ABDOMINAL	
9. AFTER TRACING EXPOSED ANIMALS		9. HEAD & THORACIC & ABDOMINAL		
0. OTHER (Miscellaneous)				

SECTION I - OWNERSHIP AND IDENTIFICATION OF REACTOR(S) PURCHASED FROM A KNOWN SOURCE

NAME OF OWNER OF INFECTED HERD	ADDRESS	1	NAME (2-7)	COUNTY (8-10)	STATE (11-12)
		3			
COUNTY	STATE	OWNER CLASS (Check one) (13)		DATE TEST READ (Mo., day, yr.) (14-19)	REASON FOR TEST (20)
		1 FARMER	3 STOCKYARD		HERD LESION CODE (21)
		2 DEALER	4 SALES RING		

The following animal reacted to the tuberculin test, and was reported to have been purchased from the previous owner named below. All herds with which this animal may have associated previously should be investigated for evidence of TB.

IDENTIFICATION TAG OR TATTOO (22-30)	REACTOR TAG	AGE (31-32) (33)	BREED (34)	SEX (34)	ORIGIN REACTOR	LESION (35)	LAB RESULTS (36)	DESCRIPTION (Color, markings, horn) (Registration No. and name if purebred)	DATE PURCHASED	
									MONTH (37-38)	YEAR (39-40)
				4						

SECTION II - PREVIOUS OWNER OF ANIMAL FOUND TO BE A REACTOR

PURCHASED FROM (Hard to be investigated)	ADDRESS		
COUNTY	STATE	OWNER CLASS (Check one)	
		1 FARMER	3 STOCKYARD
		2 DEALER	4 SALES RING

SECTION III - INTERSTATE MOVEMENT (Furnish the following information when interstate movement is involved)

1. SERIAL NUMBER, DATE, CONSIGNOR'S NAME AND ADDRESS, AND OTHER PERTINENT INFORMATION SHOWN ON THE HEALTH CERTIFICATE COVERING THE ANIMAL WHEN MOVED INTERSTATE. (If the health certificate cannot be located, a statement to that effect should be made.)	
2. IF THE ANIMAL WAS RETAGGED AFTER LEAVING THE STATE OF ORIGIN - LIST THE ORIGINAL TAG NUMBER IF KNOWN, OR GIVE ANY OTHER PERTINENT INFORMATION THAT MAY MAKE TRACING POSSIBLE.	
3. ALL TEST DATES AND RESULTS OF HERD TESTS IN WHICH THE SUBJECT ANIMAL WAS A MEMBER AFTER IT HAS ENTERED THE RECEIVING STATE. (If no tests have been made, it should be so indicated.)	

4. DISTRIBUTION: 1 COPY - VETERINARIAN IN CHARGE; 1 COPY - VS, HYATTSVILLE, MD.; 1 COPY - STATE VETERINARIAN		
REPORT SENT TO OFFICIALS IN STATE OF ORIGIN OR INSPECTOR	SIGNATURE OF VETERINARIAN IN CHARGE	DATE

VS FORM 6-4A REPLACES ANH FORM 6-4A, DEC. 1969, WHICH CAN BE USED UNTIL EXHAUSTED. JULY 1973

Brucellosis and Bovine Tuberculosis Program Standards

SECTION IV - REPORT OF RESULTS OF INVESTIGATION OF TUBERCULOSIS

PREVIOUS OWNER (41)			ADDRESS				NAME (42-47)		COUNTY (48-50)	STATE (51-52)	
1 COUNTY			STATE				OWNER CLASS (53) (Check one)		HOW WAS REACTOR LISTED IN PART I ACQUIRED BY THIS OWNER? (Check one) (54)		DATE PURCHASED (Mo., yr.) (55-58)
			1 FARMER		3 STOCK-YARD		1 RAISED ON FARM		3 PURCHASED UNK. SOURCE		
			2 DEALER		4 SALES RING		2 ORIGIN UNKNOWN		4 PURCHASED KNOWN SOURCE		
HERD TEST RESULTS	DATE TEST READ			NUMBER OF		HERD TEST LESION CODE (70)	NUMBER OF REACTORS				REMARKS CODE (79-80)
	MONTH (59-60)	DAY (61-62)	YEAR (63-64)	ANIMALS TESTED (65-67)	REACTORS (68-69)		NO P.M. REPORT (71)	NGL (72-74)	SKIN (75-76)	INTERNAL LESION (77-78)	
REMARKS (Reason test not made on this herd)											

PREVIOUS OWNER (41)			ADDRESS				NAME (42-47)		COUNTY (48-50)	STATE (51-52)	
2 COUNTY			STATE				OWNER CLASS (53) (Check one)		HOW WAS REACTOR LISTED IN PART I ACQUIRED BY THIS OWNER? (Check one) (54)		DATE PURCHASED (Mo., yr.) (55-58)
			1 FARMER		3 STOCK-YARD		1 RAISED ON FARM		3 PURCHASED UNK. SOURCE		
			2 DEALER		4 SALES RING		2 ORIGIN UNKNOWN		4 PURCHASED KNOWN SOURCE		
HERD TEST RESULTS	DATE TEST READ			NUMBER OF		HERD TEST LESION CODE (70)	NUMBER OF REACTORS				REMARKS CODE (79-80)
	MONTH (59-60)	DAY (61-62)	YEAR (63-64)	ANIMALS TESTED (65-67)	REACTORS (68-69)		NO P.M. REPORT (71)	NGL (72-74)	SKIN (75-76)	INTERNAL LESION (77-78)	
REMARKS (Reason test not made on this herd)											

PREVIOUS OWNER (41)			ADDRESS				NAME (42-47)		COUNTY (48-50)	STATE (51-52)	
3 COUNTY			STATE				OWNER CLASS (53) (Check one)		HOW WAS REACTOR LISTED IN PART I ACQUIRED BY THIS OWNER? (Check one) (54)		DATE PURCHASED (Mo., yr.) (55-58)
			1 FARMER		3 STOCK-YARD		1 RAISED ON FARM		3 PURCHASED UNK. SOURCE		
			2 DEALER		4 SALES RING		2 ORIGIN UNKNOWN		4 PURCHASED KNOWN SOURCE		
HERD TEST RESULTS	DATE TEST READ			NUMBER OF		HERD TEST LESION CODE (70)	NUMBER OF REACTORS				REMARKS CODE (79-80)
	MONTH (59-60)	DAY (61-62)	YEAR (63-64)	ANIMALS TESTED (65-67)	REACTORS (68-69)		NO P.M. REPORT (71)	NGL (72-74)	SKIN (75-76)	INTERNAL LESION (77-78)	
REMARKS (Reason test not made on this herd)											

REPORT OF RESULTS OF INVESTIGATION SENT TO VETERINARY SERVICES, HYATTSVILLE, MD.	DATE
SIGNATURE OF VETERINARIAN IN CHARGE	

Appendix 4 C: Animal Trace-in Documentation: Tuberculosis

VS Form 35-B

CLOSING REPORT OF HERD INVESTIGATION OF TUBERCULOSIS LESIONS OR THORACIC ORANULOMAS IN REGULAR KILL ANIMALS			1. ESTABLISHMENT & CASE NO.		2. STATE CASE NO.				
3. HISTOPATHOLOGY		4. BACTERIOLOGY		5. LAB ACCESSION NO.		6. SLAUGHTER DATE			
7. FIELD INVESTIGATION REPORT REQUIREMENTS <ul style="list-style-type: none"> Full information relating to source of the subject animal; name of agencies such as owners, dealers, auction markets, or previous herd owner that may have handled the animal. Approximate date of transfers should be shown. Diagram all movement. Brief history of recent tuberculin tests of each herd with which the infected animal has been associated. Show whether animals being traced were included in these tests. Tests performed prior to the date of the VS 6-35 are not acceptable for purposes of closing this investigation. How information as ascertained or verified (attach VS 6-35A and appropriate diagram of movements) 									
COMMENTS									
8. HERDS TESTED			CODE (Enter in Col. J) S- State Employed C- County Employed F- Federally Employed A- Accredited						
NAME OF HERD OWNER	COUNTY	DATE TESTED	NUMBER			POST MORTEM		TESTING VETERINARIAN	
			TESTE D	REAC- TORS	SUS- PECTS	NO. W/ LESION	NO. NGL	NAME	COD E
A	B	C	D	E	F	G	H	I	J

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VS Form 6-4 should be submitted for each herd with lesioned reactors									
9. SIGNATURE OF VETERINARIAN IN CHARGE					10. STATION			11. DATE	
VS FORM 6-35B								USDA - APHIS	

Appendix 5 A: Animal Trace-out Documentation: Brucellosis

VS Form 4-108B

U.S. DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE VETERINARY SERVICES ANIMALS REMOVED FROM INFECTED HERD SUPPLEMENTAL TO VS FORM 4-108		INSTRUCTIONS: List all animals (include feeder heifers and slaughtered cows and bulls but exclude reactors) sold or otherwise removed from the herd within the last two years, or since infection was introduced. Obtain all supporting documents.	1. NAME OF HERD OWNER 3. COUNTY	2. DOCUMENT NUMBER 4. HERD NO.	5. PAGE OF PAGES										
6. LIST OF ANIMALS SOLD OR OTHERWISE REMOVED FROM THE HERD															
BUYER OR RECIPIENT		SALES		IDENTIFICATION							REMARKS (Stage of pregnancy, etc.)	BRUCELLOSIS STATUS			
NAME AND ADDRESS A		Date B	No. of Animals C	Tag No's D	Other ID (Brands, Tattoos, etc.) E	Sex F	Age Sold G	When Brought H	Vacc. Status I	Disposition J		Vacc. (see code) K	L	1 - Untested	2 - Tested Neg. M

Brucellosis and Bovine Tuberculosis Program Standards

NOTICE TO HERD OWNERS

Herd Owners (*Managers or Agents*) of herds of brucellosis affected livestock must be advised that information is given voluntarily to assist in the elimination of brucellosis from the livestock population. Cooperation of all affected herd owners (*Managers or Agents*) is needed to complete a thorough epidemiologic investigation to identify the source of the disease, the method of spread and the possible dissemination to new herds. The authorities under which the brucellosis program is conducted are contained in 21 USC 111, 112, 114, 114a-1, 115, 120, 121, 125, and 134a-f and Title 9, Code of Federal Regulations, Part 51 and Part 78.

INSTRUCTIONS

The purpose of this form is to record information of epidemiologic importance for each potentially brucella exposed animal removed from this herd. This information will facilitate tracing of each animal for retesting or for verification of slaughter.

- 1-4. From 4-108. Item 2 is the preprinted number of the 4-108.
5. Indicate which page of the 4-108B this is and how many total pages were completed.
6. List each animal (*except steers or reactors*) removed from the herd for any reason since it became infected (*if this date can be established*) or otherwise for the 2-year period prior to discovery of infection.
- 6-A. Record the full legal name and address of the recipient of each animal. Each animal should be listed individually if individual identification is available, otherwise they may be listed by transaction lots.
- 6-B. Record the date this herd owner gave up control of the animals.
- 6-C. Indicate the number of animals involved with each transaction.
- 6-D. Record all available identification at time of sale (*removal & E. from premises*) for each animal including eartags, salestags, backtags, bangle tags, brands, identification tattoos, and registration numbers.
- 6-F. Enter F (*female*) or M (*male*). If a lot, indicate the number of each.
- 6-G. Age at time of removal from herd. If a lot, indicate the average or predominant age.
- 6-H. Indicate the specific breed. If nondescript, indicate the breed that the animal most appears to represent. For lots, enter the predominant breed or record as mixed beef, etc.
- 6-I. Record the vaccination status as "CV", "AV", "NV" (*not vaccinated*) or "UN" (*unknown*) and list the vaccination tattoo if applicable.
- 6-J. Indicate disposition of animals by recording "S" for slaughter, "F" for feedlots, veal feeding, dairy heifer lot, etc., and "H" for herd replacement.
- 6-K. Indicate the status of the tracing on each animal by 1 (*slaughter verified*), 2 (*located and retested*), 3 (*located but not tested*), 4 (*recipient state notified*), or 5 (*not verified*).
- 6-L. Record the stage of pregnancy and other pertinent information as of the time of sale. Also list the buyer if different than that given in A.
- 6-M. List status of animal when it left the herd or first point of concentration.
7. Indicate if arrangements have been made to contact everyone that received animals from this herd.
- 8-9. Self-explanatory.

Submit copies of any documents that may help trace the animals such as invoices, brand inspection, cancelled checks, etc.

Appendix 5 B: Animal Trace-out Documentation: Tuberculosis

Print Form

USDA-APHIS

INVESTIGATION FOR EVIDENCE OF TUBERCULOSIS *(Exposed Animal)*

CODES - *(The following codes are to be used in the respective blocks in Section I.)*

REASON FOR TEST <i>(Block 4)</i>	REACTOR LESION <i>(Block 6)</i>	BREED <i>(Block 10)</i>	SEX <i>(Block 11)</i>
1. Area 2. Herd Accreditation or Reaccreditation 3. Comply with Milk Ordinances 4. Sale, Show, Interstate, Intra-State or Export Shipment 5. Imported Animals <i>(Interstate or International)</i> 6. Retest of Quarantined Herd 7. After Tracing Animals with Lesions Regular Kill <i>(VS FORM 6-35)</i> 8. After Tracing Reacting Animals <i>(VS FORM 6-4A)</i> 9. After Tracing Exposed Animals <i>(VS FORM 6-4B)</i> 0. Other <i>(Miscellaneous)</i>	0. No P. M. RPT. 1. NGL 2. Skin 3. Head 4. Thoracic 5. Abdominal 6. Head and Thoracic 7. Head and Abdominal 8. Thoracic and Abdominal 9. Head and Thoracic and Abdominal	1. Holstein 2. Hereford 3. Jersey 4. Guernsey 5. Angus 6. Ayrshire 7. Shorthorn 8. Swiss 9. Other	1. Female 2. Male 3. Steer
		ANIMALS SOLD FOR <i>(Block 12)</i> 1. Dairy 2. Breeding 3. Feeding 4. Slaughter	RAISED ON FARM <i>(Block 13)</i> 1. Yes 2. No

SECTION I - IDENTIFICATION OF EXPOSED ANIMAL SOLD FROM INFECTED HERD

1. NAME AND ADDRESS OF OWNER OF INFECTED HERD <i>(Include State and ZIP Code)</i>			2. COUNTY <i>(In which herd is located)</i>		
3. DATE TEST READ <i>(Mo., Day, Year)</i>	4. REASON(S) FOR TEST <i>(See codes above)</i>	5. OWNER CLASS <i>("X" as appropriate)</i>		6. HERD LESION CODE <i>(See codes above)</i>	
		(1) Farmer	(3) Stockyard		
		(2) Dealer	(4) Sales Ring		

The animal identified below was exposed to reactors in the herd indicated above and was sold to the herd owner indicated in Section II. Evidence suggests that the subsequent owner's herd should be investigated for possible tuberculosis.

7. IDENTIFICATION TAG OR TATTOO	8. SALES - MARKET - RETAINED TAG	9. AGE	<i>(See codes above)</i>				14. DATE SOLD	
			10. BREED	11. SEX	12. SOLD FOR	13. RAISED ON FARM	Month	Year
15. DESCRIPTION <i>(Color, markings, horns) (Registration No. and name if purebred)</i>								

SECTION II - SUBSEQUENT OWNER OF EXPOSED ANIMAL

16. TO WHOM SOLD <i>(To be investigated)</i>			17. ADDRESS				
18. COUNTY		19. STATE		20. OWNER CLASS <i>("X" as appropriate)</i>			
				(1) Farmer	(3) Stockyard		
				(2) Dealer	(4) Sales Ring		

SECTION III - INTERSTATE MOVEMENT *(Furnish the following information when interstate movement is involved)*

21. Serial number, date, consignor's name and address, and other pertinent information shown on the health certificate covering the animal when moved interstate. <i>(If the health certificate cannot be located, make a statement to that effect.)</i>	
22. All test dates and results of herd tests in which the subject animal was a member before it entered the receiving state. <i>(If no tests have been made, make a statement to that effect.)</i>	

REMARKS: *(Cite item number referred to.)*

23. REPORT SENT TO OFFICIALS IN RECIPIENT STATE <i>(indicate State)</i>	24. SIGNATURE OF VETERINARIAN IN CHARGE	25. DATE
i		

COPY DISTRIBUTION: Original and one copy to Regional Epidemiologist (original to be forwarded to Hyattsville, copy to be retained); one copy to each Veterinarian In Charge involved; one copy to be retained by originator.

VS FORM 6-4B *Previous editions are obsolete*
(JUL 82)

(See Reverse)

Brucellosis and Bovine Tuberculosis Program Standards

SECTION IV - REPORT OF RESULTS OF INVESTIGATION AFTER TRACING AN EXPOSED ANIMAL

1. NAME AND ADDRESS OF SUBSEQUENT OWNER (Include State and ZIP Code)	A. COUNTY	B. ANIMAL IDENTIFICATION	C. DATE TEST READ (Mo., Day, Yr.)	D. NO. OF:				E. NO. OF REACTORS-- WITH LEASONS				F. Is the exposed animal still in the herd? <input type="checkbox"/> yes, if not, sold for: ("X" as appropriate)				G. DATE SOLD (Mo. & Yr.)	H. OWNER CLASS ("X" as appropriate)			
				(1) ANIMALS TESTED	(2) REACTORS	(1) NO. P. M. REPORT	(2) NGL	(3) SKIN	(4) INTER-INTERNAL TUBSON	(1) DAIRY	(2) BREEDING	(3) SLAUGHTER	(4) FEEDING	(1) FARMER	(2) DEALER		(3) STOCK-YARD	(4) SALES RING		
REMARKS: (Reason(s) test not made on this herd)																				
2.																				
REMARKS: (Reason(s) test not made on this herd)																				
3.																				
REMARKS: (Reason(s) test not made on this herd)																				
4.																				
REMARKS: (Reason(s) test not made on this herd)																				
5.																				
REMARKS: (Reason(s) test not made on this herd)																				
6.																				
REMARKS: (Reason(s) test not made on this herd)																				
7.																				
REMARKS: (Reason(s) test not made on this herd)																				
STATE(S) CASE TRANSFERRED TO	REPORT OF RESULTS OF INVESTIGATION SENT TO VETERINARY SERVICES, HYATTSVILLE, MD. SIGNATURE OF VETERINARIAN IN CHARGE															DATE				
DISTRIBUTED TO ("X" as appropriate)	HYATTSVILLE <input type="checkbox"/>	REGIONAL DIRECTOR <input type="checkbox"/>	REGIONAL EPIDEMIOLOGIST <input type="checkbox"/>	STATE OF ORIGIN (If interested) <input type="checkbox"/> State <input type="checkbox"/> Federal				STATE OF DESTINATION (If transferred) <input type="checkbox"/> State <input type="checkbox"/> Federal												

Appendix 6 A: Affected Herd Report Template: Brucellosis

(See Notice/Instructions on reverse)

Form Approved OMB NO. 40-R4028

U.S. DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE VETERINARY SERVICES RCS # 34-VS-71				FOR OFFICE USE ONLY				
EPIDEMIOLOGIC INVESTIGATION OF BRUCELLOSIS REACTOR HERD				Assigned to Veterinarian: _____ Code: _____ Date Assigned _____				
1. Name of Herd Owner _____ 2. Street Address _____				Date of Current Test _____	TEST RESULTS (No. of Cattle)			
3. City and State (Include Zip Code) _____				Prior history of Brucellosis in herd <input type="checkbox"/> Yes <input type="checkbox"/> No	Total	Negative	Suspect	Reactor
4. County _____ 5. RGE _____ TWP _____ SEC _____ 6. Herd No. _____					Total No. Reactors found	Date Reactors last found	Other States Involved have been notified <input type="checkbox"/> Yes <input type="checkbox"/> No	
7. Reason for Test <input type="checkbox"/> Slaughter Reactor <input type="checkbox"/> Diagnostic (Abortion, Etc.) <input type="checkbox"/> Post Movement Retest <input type="checkbox"/> Epid. (Tracebacks from infected herds, adjacent herds, sales, neighborhood herds, or contact herds on common pasture). <input type="checkbox"/> Other (specify below) <input type="checkbox"/> Livestock Market Reactor <input type="checkbox"/> Private sale or show <input type="checkbox"/> Area Test (Community test in heavily infected area or area-wide recertification tests). <input type="checkbox"/> Brucellosis Ring Test <input type="checkbox"/> Herd Certification Test				Reviewed by Epidemiologist: _____ Date Reviewed _____				
8. Are Clinical Signs of Brucellosis Present? (If yes, describe signs)				9. Percentage of herd Vaccinated for Brucellosis CV _____ % AV _____ %				
A. Human <input type="checkbox"/> Yes <input type="checkbox"/> No B. Animal <input type="checkbox"/> Yes <input type="checkbox"/> No C. Animal Clinical Signs <input type="checkbox"/> Abortion No. _____ <input type="checkbox"/> Hygromas No. _____ <input type="checkbox"/> Ret. Placenta No. _____ <input type="checkbox"/> Reduced Milk Prod. No. _____ <input type="checkbox"/> Weak Calves No. _____ <input type="checkbox"/> Fistulous Withers/Poil Evil (Horses) No. _____				10. Maximum age when calfhood vaccinated _____				
HERD STATUS	11. No. of Herds Owned or Managed _____				12. Location of Herd (Continue on separate page)			
	A. _____				13. Date Test Scheduled _____			
	B. _____				14. If all herds are not to be tested, give reason			
	C. _____							
HERD	15. Type of Operation(s)				16. Cattle Census on Premises (Exclude steers and spayed heifers)			
	Dairy	Beef	Feed-lot	Cows Vac	Cows Non Vac	Bulls	Heifers 1 to 2 years Vac	Heifers 1 to 2 years Non Vac
							Heifers under 12 months Vac	Heifers under 12 months Non Vac
	A. _____							
17. No. Susceptible Species on Premises				18. Breeding Program this Herd (Check one in column A.)				
Swine	Goats	Sheep	Horses	Buffalo	Dogs	Other (Specify species and no.)	A. <input type="checkbox"/> Natural <input type="checkbox"/> Art. Insem.	B. Dates of Usual Calving Season: Beginning Month _____ Ending Month _____
19. Owner's opinion on source of infection				20. In my opinion this herd is infected with Brucellosis <input type="checkbox"/> Yes <input type="checkbox"/> No				
21. Probable source of infection (Specify)				22. Date infection introduced into herd				
23. Origins of this herd (All raised, recently assembled, few purchased additions, many purchased additions)				24. Cattle moved out of herd since date infection introduced <input type="checkbox"/> Yes <input type="checkbox"/> No (If Yes, complete VS 4-108B)				
25. Reported sales "to slaughter" verified <input type="checkbox"/> Yes <input type="checkbox"/> No Assistance needed to verify slaughter				26. Other sales verified <input type="checkbox"/> Yes <input type="checkbox"/> No				
COMPLETE ITEMS 27 and 28 WHERE APPLICABLE AND COMPLETE VS FORM 4-108A								
TRACEBACK AND CONTACT INFORMATION	27. Reactors were raised <input type="checkbox"/> Yes <input type="checkbox"/> No				28. A. Where obtained B. No. obtained C. Date obtained D. Accompanied by Health Certificate, Blood Test Record, or Pern			
	(If no, complete Item 28.)				Yes _____ None _____ Some but not all _____			
					(Attach copy of bill of sale, health certification, etc., if possible)			
	29. LIST NAMES OF SIX NEAREST HERD OWNERS AND COMPLETE VS FORM 4-108C							
(1) _____		(2) _____		(3) _____				
(4) _____		(5) _____		(6) _____				
30. Quarantine and requirements for quarantine release have been explained to owner <input type="checkbox"/> Yes <input type="checkbox"/> No				31. Permit requirements and requirements for the Hot "S" Brand on exposed animals before movement into market channels explained to owner <input type="checkbox"/> Yes <input type="checkbox"/> No				
32. Explained the nature of Brucellosis, discussed a tentative retest schedule, and completed Herd Plan has been submitted. <input type="checkbox"/> Yes <input type="checkbox"/> No (If no, explain in item 36)				33. Anticipated owner cooperation <input type="checkbox"/> Good <input type="checkbox"/> Average <input type="checkbox"/> Poor				
34. Owner knows how to contact you? <input type="checkbox"/> Yes <input type="checkbox"/> No								
Supplemental forms completed <input type="checkbox"/> VS Form 4-108A <input type="checkbox"/> VS Form 4-108B <input type="checkbox"/> VS Form 4-108C				36. REMARKS (Attach supplemental sheet if necessary. Cite item number referred to.)				
37. Date quarantine _____		38. Quarantine No. _____		39. Signature of VMO _____		Code: _____ 40. Date Signed _____		

VS Form 4-108

VS FORM 4-108 Previous editions are obsolete. (OCT 80)

HERD FILE

Brucellosis and Bovine Tuberculosis Program Standards

NOTICE TO HERD OWNERS

Herd Owners (*Managers or Agents*) of herds of brucellosis affected livestock must be advised that information is given voluntarily to assist in the elimination of brucellosis from the livestock population. Cooperation of all affected herd owners (*Managers or Agents*) is needed to complete a thorough epidemiologic investigation to identify the source of the disease, the method of spread and the possible dissemination to new herds. The authorities under which the brucellosis program is conducted are contained in 21 USC 111, 112, 114, 114a, 114a-1, 115, 120, 121, 125, and 134a-f and Title 9, Code of Federal Regulations, Part 51 and 78.

INSTRUCTIONS

(For Complete Instructions see VS Memorandum 551.26)

1. **COMPLETE LEGAL NAME** as used on indemnity papers.
- 2-3. **COMPLETE MAILING ADDRESS** including post office box number, route number, and zip code.
4. List county in which herd is located.
5. Geographic location of farm where subject animals are located -- use range, township and section, or longitude and latitude coordinates or mileage grid indicating distance north and west from southeast corner of county -- use only one system within a State.
6. To be completed in office unless herd number is known.
7. Reasons for test:
 - Slaughter Reactor** - MCI reactor disclosed at a slaughter plant.
 - Livestock Market Reactor** - MCI reactor disclosed at a livestock market.
 - Brucellosis Ring Test** - Herd Test because of suspicious milk test.
 - Diagnostic** - Abortion, infertility, etc.
 - Private sale or show** - Cross out nonapplicable item.
 - Herd Certification Test** - Initial or recertification tests.
 - Post-Movement Retests** - Test performed after purchase for cattle moved under permit and held under quarantine for retest.
 - Area Test** - (*Community test in heavily infected area or area-wide recertification tests*) - Cross out nonapplicable item.
 - Epidemiologic** - (*Tracebacks from infected herds, i.e., cattle were sold from this herd into an infected herd; adjacent or fence contact herds, sales, i.e., cattle were purchased from an infected herd, neighborhood herds, or contact herds on common premises*). Cross out nonapplicable categories.
 - Other** - (*Specify*) - Any tests not covered by the above categories.
8. Report number of animals observed by owner or others showing clinical signs since estimated onset of infection (see item 22).
9. Estimate percentage of animals in herd that were vaccinated in calfhood or as adults. If calfhood vaccinated animals are revaccinated as adults, make a notation of this in remarks (36).
10. The age of the oldest calf at time of vaccination should be recorded in months.
- 11-16. Report the total number of separate (*by UM&R definition*) herds owned and the number of cattle in each. Prepare a separate 4-108 for each herd listed and cross-reference all reports (*forms*). Specialized operations such as veal raising or dairy heifers should be included under feedlots (15) and described under remarks.
18. If more than one term is applicable in block A, give the percentage of each. In block B, indicate beginning and ending month of calving season.
21. Specify the name of herd owner if known and probable method of spread (*e.g., area spread, purchased animal, common range, etc.*).
22. Estimate from epidemiological information the probable date that brucellosis was introduced into the herd.
24. Include all cattle, other than steers or spayed heifers, moved for any purpose. This includes day-old calves, cull cows, feeder heifers, etc.
25. Verify reported sales to slaughter by checking purchase and sales receipts at markets (*or dealer*) and purchase receipts at slaughter plants.
26. Verify by locating and retesting the animal(s) or by notifying state of destination.
27. If any reactors were not raised in the herd, the response is "NO".
28. Give information on the purchase lot(s) (*summarize for each category*) from which reactors originated.
29. List the six nearest herds regardless of distance. If more than six herds have potential contact, give details on separate sheets including locations. Potential contact means epidemiological possibility of exposure and includes indirect as well as direct contact.
36. A narrative statement of your appraisal of the situation should be attached.

Appendix 6 B: Affected Herd Report Template: Tuberculosis

VS Form 6-4

USDA-APHIS REPORT ON HERD REVEALING REACTORS TO TUBERCULIN TEST																															
NAME OF HERD OWNER		ADDRESS		OWNER (2-7)		COUNTY STATE		STATE CODE (11-12)																							
				1 1																											
				COUNTY				OWNER CLASS (Check one) (13)																							
								1 FARMER 3 STOCKYARD																							
								2 DEALER 4 SALES RING																							
<p>REASON FOR TEST (20)</p> <p>1. AREA</p> <p>2. HERD ACCREDITATION OR REACCREDITATION</p> <p>3. COMPLY WITH MILK ORDINANCES</p> <p>4. SALE, SHOW, INTERSTATE, INTRA-STATE OR EXPORT SHIPMENT</p> <p>5. IMPORTED ANIMALS (Interstate or International)</p> <p>6. RETEST OF QUARANTINED HERD</p> <p>7. AFTER TRACING ANIMALS WITH LESIONS</p> <p>8. REGULAR KILL</p> <p>9. AFTER TRACING REACTING ANIMALS</p> <p>9. AFTER TRACING EXPOSED ANIMALS</p> <p>0. OTHER (Miscellaneous)</p>																															
<p>HERD TEST LESION CODE (21)</p> <p>1. DAIRY CATTLE</p> <p>2. BEEF CATTLE</p> <p>3. DAIRY AND BEEF</p> <p>4. FIELD</p> <p>5. INJECTION (27)</p> <p>6. CAUDAL</p> <p>7. VULVA</p> <p>8. CAUDAL AND VULVA</p> <p>9. CAUDAL AND CERVICAL</p> <p>0. VULVA AND CERVICAL</p> <p>7. CAUDAL YULVA & CERVICAL</p>																															
<p>TYPE OF CATTLE (26)</p> <p>1. FEDERAL</p> <p>2. STATE</p> <p>3. MUNICIPAL</p> <p>4. FIELD</p> <p>5. LAB RESULTS (63)</p> <p>6. COMPATIBLE</p> <p>7. SUGGESTIVE</p> <p>8. NOT SUGGESTIVE</p> <p>9. NO SPECIMEN</p>																															
<p>POST MORTEM (62)</p> <p>1. FEDERAL</p> <p>2. STATE</p> <p>3. MUNICIPAL</p> <p>4. FIELD</p> <p>5. LAB RESULTS (63)</p> <p>6. COMPATIBLE</p> <p>7. SUGGESTIVE</p> <p>8. NOT SUGGESTIVE</p> <p>9. NO SPECIMEN</p>																															
<p>ACTION TAKEN (72)</p> <p>1. HERD PLACED IN QUARANTINE</p> <p>2. HERD RELEASED FROM QUARANTINE</p> <p>3. SUSPECTS RELEASED FROM QUARANTINE</p> <p>4. SUSPECTS QUARANTINED</p> <p>5. CONTINUE HERD QUARANTINE</p> <p>6. NO ACTION TAKEN</p> <p>7. ENTIRE HERD SLAUGHTERED</p>																															
<p>SECTION I - HERD HISTORY (Start with current test and work back)</p>																															
DATE TEST READ	YEAR	MONTH	REASON FOR TEST	HERD LESION CODE	CURRENT AND/OR PREVIOUS TEST NO.	PROGRAM-TEST CODE	VETERINARIAN LAST NAME	OFFICIAL STATUS	TYPE OF CATTLE	INJECTION	NUMBER OF ANIMALS		NUMBER OF REACTORS	NUMBER OF SUSPECTS	NO GROSS LESIONS	NUMBER OF REACTORS SHOWING INTERNAL LESIONS BY LOCATION				POST MORTEM	LAB RESULTS	ORIGIN OF REACTORS				ACTION TAKEN					
											IN HERD	TESTED				0	1	2	3			4	5	6	7		8	9	1	2	3
14-18	10-17	10-19	20	21	22	23-24					28-31	32-35	36-38	39-41	42	43-45	46-47	48-49	50-51	52-53	54-55	56-57	58-59	60-61	62-63	64-65	66-67	68-69	70-71	72	73
					1																										
					2																										
<p>REMARKS (Use reverse side of this form if additional space is needed)</p>																															
																				7#00											
DATE PREVIOUS VS FORM 6-4 REPORT WAS SUBMITTED										SIGNATURE OF VETERINARIAN IN CHARGE										DATE											

VS FORM 6-4 (NOV 73)

Appendix 6 C: Affected Herd Report Template: Tuberculosis

VS Form 6-22A

According to the Paperwork Reduction Act of 1985, no persons are 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 0579-0084. The time required to complete this information collection is estimated to average 1.3 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. **FORM APPROVED - OMB NO. 0579-0084**

U.S. DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE VETERINARY SERVICES		TUBERCULOSIS INFECTED HERD				INSTRUCTIONS: Prepare at the time of identification of reactors, and attach to VS Form 6-22, Tuberculosis Test Record.			
		<input type="checkbox"/> BOVINE <input type="checkbox"/> CERVINE <input type="checkbox"/> OTHER							
NAME OF OWNER OF INFECTED HERD		FARM NO.	ADDRESS OF OWNER			OFFICE USE			
COUNTY		OWNER CLASS (Check one)			DATE TEST READ (Month, day, year)	HERD LESION CODE	LAB RESULTS		
		1 FARMER	3 STOCKYARD						
		2 DEALER	4 SALES RING						
SECTION I - OTHER ANIMALS ON FARM (Inventory; use continuation sheet if needed)									
LIST SPECIES (Specify e.g. swine, poultry, cervid, llama, antelope, etc.)	CONTACT WITH REACTOR	NUMBER			NECOPSIED				
		YES	NO	ON THE FARM	TESTED	REACTED	NUMBER	NO. WITH TB	
SECTION II - IDENTIFICATION AND HISTORY OF REACTORS FOUND ON THIS TEST									
IDENTIFICATION TAG OR TATTOO	REACTOR TAG	AGE	BREED	SEX	ORIGIN OF REACTOR		DESCRIPTION (Registration No., color, markings, etc.)	LESION CODE	LAB RESULTS
					1 RAISED ON FARM	3 PURCHASED UNKNOWN SOURCE			
					2 ORIGIN UNKNOWN	4 PURCHASED KNOWN SOURCE			
1 PURCHASE FROM		ADDRESS			OWNER CLASS (Purchased from)		DATE PURCHASED		
					1 FARMER	3 STOCK-YARD	MONTH	YEAR	
					2 DEALER	4. SALES RING			
IDENTIFICATION TAG OR TATTOO	REACTOR TAG	AGE	BREED	SEX	ORIGIN OF REACTOR		DESCRIPTION (Registration No., color, markings, etc.)	LESION CODE	LAB RESULTS
					1 RAISED ON FARM	3 PURCHASED UNKNOWN SOURCE			
					2 ORIGIN UNKNOWN	4 PURCHASED KNOWN SOURCE			
2 PURCHASE FROM		ADDRESS			OWNER CLASS (Purchased from)		DATE PURCHASED		
					1 FARMER	3 STOCK-YARD	MONTH	YEAR	
					2 DEALER	4. SALES RING			
IDENTIFICATION TAG OR TATTOO	REACTOR TAG	AGE	BREED	SEX	ORIGIN OF REACTOR		DESCRIPTION (Registration No., color, markings, etc.)	LESION CODE	LAB RESULTS
					1 RAISED ON FARM	3 PURCHASED UNKNOWN SOURCE			
					2 ORIGIN UNKNOWN	4 PURCHASED KNOWN SOURCE			
3 PURCHASE FROM		ADDRESS			OWNER CLASS (Purchased from)		DATE PURCHASED		
					1 FARMER	3 STOCK-YARD	MONTH	YEAR	
					2 DEALER	4. SALES RING			
IDENTIFICATION TAG OR TATTOO	REACTOR TAG	AGE	BREED	SEX	ORIGIN OF REACTOR		DESCRIPTION (Registration No., color, markings, etc.)	LESION CODE	LAB RESULTS
					1 RAISED ON FARM	3 PURCHASED UNKNOWN SOURCE			
					2 ORIGIN UNKNOWN	4 PURCHASED KNOWN SOURCE			
4 PURCHASE FROM		ADDRESS			OWNER CLASS (Purchased from)		DATE PURCHASED		
					1 FARMER	3 STOCK-YARD	MONTH	YEAR	
					2 DEALER	4. SALES RING			
IDENTIFICATION TAG OR TATTOO	REACTOR TAG	AGE	BREED	SEX	ORIGIN OF REACTOR		DESCRIPTION (Registration No., color, markings, etc.)	LESION CODE	LAB RESULTS
					1 RAISED ON FARM	3 PURCHASED UNKNOWN SOURCE			
					2 ORIGIN UNKNOWN	4 PURCHASED KNOWN SOURCE			
5 PURCHASE FROM		ADDRESS			OWNER CLASS (Purchased from)		DATE PURCHASED		
					1 FARMER	3 STOCK-YARD	MONTH	YEAR	
					2 DEALER	4. SALES RING			

VS FORM 6-22A (FEB 99) (Previous editions are obsolete.) (Over)

Brucellosis and Bovine Tuberculosis Program Standards

SECTION III - IDENTIFICATION OF EXPOSED ANIMALS SOLD FROM THIS HERD PRIOR TO THIS REPORT <i>(List all cattle that have been sold from this herd for purposes other than immediate slaughter to known buyers)</i>										
IDENTIFICATION TAG OR TATTOO	SALES TAG	AGE	BREED	SEX	SOLD FOR				RAISED ON FARM	DESCRIPTION (Registration No., color, markings, etc.)
					1 DAIRYING		3 FEEDING			
					2 BREEDING		4 SLAUGHTER			
TO WHOM SOLD		ADDRESS			OWNER CLASS (Sold to)				DATE SOLD	
					1 FARMER		3 STOCK-YARD		MONTH	YEAR
					2 DEALER		4 SALES RING			
IDENTIFICATION TAG OR TATTOO	SALES TAG	AGE	BREED	SEX	SOLD FOR				RAISED ON FARM	DESCRIPTION (Registration No., color, markings, etc.)
					1 DAIRYING		3 FEEDING			
					2 BREEDING		4 SLAUGHTER			
TO WHOM SOLD		ADDRESS			OWNER CLASS (Sold to)				DATE SOLD	
					1 FARMER		3 STOCK-YARD		MONTH	YEAR
					2 DEALER		4 SALES RING			
IDENTIFICATION TAG OR TATTOO	SALES TAG	AGE	BREED	SEX	SOLD FOR				RAISED ON FARM	DESCRIPTION (Registration No., color, markings, etc.)
					1 DAIRYING		3 FEEDING			
					2 BREEDING		4 SLAUGHTER			
TO WHOM SOLD		ADDRESS			OWNER CLASS (Sold to)				DATE SOLD	
					1 FARMER		3 STOCK-YARD		MONTH	YEAR
					2 DEALER		4 SALES RING			
IDENTIFICATION TAG OR TATTOO	SALES TAG	AGE	BREED	SEX	SOLD FOR				RAISED ON FARM	DESCRIPTION (Registration No., color, markings, etc.)
					1 DAIRYING		3 FEEDING			
					2 BREEDING		4 SLAUGHTER			
TO WHOM SOLD		ADDRESS			OWNER CLASS (Sold to)				DATE SOLD	
					1 FARMER		3 STOCK-YARD		MONTH	YEAR
					2 DEALER		4 SALES RING			

SECTION IV - REMARKS

SIGNATURE OF VETERINARIAN

VETERINARIAN CODE

DATE

REVERSE

*U.S. Government Printing Office: 2004-617-755

Appendix 7: Epidemiological Situation Report Template and Instructions

Choose an item.

Epidemiological Situation Report

Initiating Event	Report Type	Date
Choose an item.	Choose an item.	

If "Affected Herd" please indicate date the herd was determined to be affected: _____

Location	State	County	Town	Zip Code	Area Status

Herd Type	Number of Animals	0 - 2 Months	2- 6 Months	6 - 24 Months	> 24 Months	Total
Choose an item.						

Narratives:

*NOTE: When submitting updates to previous reports do not delete any previous information! Add new information after previous descriptions and identify it as new information. Methods of identifying new information may be different color type face, **blue recommended**, or highlighting the new text.*

Current Situation and New Information: Highlight events or findings that have not previously been reported.

Brucellosis and Bovine Tuberculosis Program Standards

Priority Issues:

Critical actions to occur in the immediate future. Provide dates, if possible.

Critical decisions that need to be made and the responsible parties. Provide deadlines, if possible.

Accomplishments:

Planned Activities:

Investigation Summary:

Source of infection identified?	Choose an item.	Choose an item.
---------------------------------	-----------------	-----------------

If "other" please describe:

Additional herd details:

Brucellosis and Bovine Tuberculosis Program Standards

Other information important to the investigation:

Choose an item. Summary:

Herd Identifier _____ (DO NOT USE HERD OWNER'S NAME.)

Date	Number tested	Number Suspect	Number Reactor	Number Necropsied	Comments

Wildlife Surveillance – planned or conducted?	Choose an item.
---	-----------------

Describe:

Brucellosis and Bovine Tuberculosis Program Standards

Within State Epidemiologically Related Herds Summary:

Herd Identifier	Date - Tested or Scheduled	Reason for test	Number tested	Number Suspect	Number Reactor	Number Necropsied
		Choose an item.				
		Choose an item.				
		Choose an item.				
		Choose an item.				
		Choose an item.				

Brucellosis and Bovine Tuberculosis Program Standards

Post Mortem Results:

Herd Identifier	Animal ID	Date Posted	Gross Necropsy	Histopath	PCR	PCR Other Positive	Culture
			Choose an item.	Choose an item.	Choose an item.		Choose an item.
			Choose an item.	Choose an item.	Choose an item.		Choose an item.
			Choose an item.	Choose an item.	Choose an item.		Choose an item.
			Choose an item.	Choose an item.	Choose an item.		Choose an item.
			Choose an item.	Choose an item.	Choose an item.		Choose an item.
			Choose an item.	Choose an item.	Choose an item.		Choose an item.
			Choose an item.	Choose an item.	Choose an item.		Choose an item.

Out of State Trace Summary:

Receiving State	Date Transmitted	Trace Type	Receiving State Action Taken (Receiving State to notify originating State of actions taken)
		Choose an item.	
		Choose an item.	
		Choose an item.	
		Choose an item.	
		Choose an item.	
		Choose an item.	

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		Choose an item.	
		Choose an item.	
		Choose an item.	
		Choose an item.	
		Choose an item.	
		Choose an item.	
		Choose an item.	

Situation Report Instructions

Drop-Down Menu Instructions

Drop-Down menus are used throughout this report. They may be found in any cell that contains the words “Choose an item.” in light gray letters. To access the drop down menus do the following:

- 1) Place the cursor on the words “Choose an item” (Figure 1 - blue arrow) and left click.
- 2) The drop down menu arrow will appear to the right of the cell (Figure 1 - red circle).
- 3) Left click the drop down menu arrow and the selections for completing the cell entry will appear (Figure 2 - green circle).
- 4) Move the cursor to the desired selection which will become highlighted and left click to complete the selection process

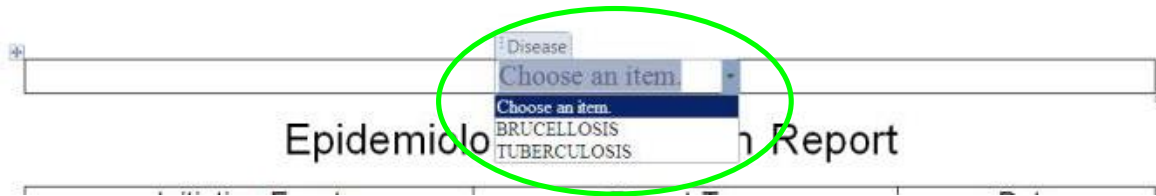
These 4 steps may be repeated anywhere a yellow highlighted cell is present.

Figure 1.

The image shows a screenshot of the 'Epidemiological Situation Report' form. At the top, there is a text input field containing the placeholder text 'Choose an item.' with a blue arrow pointing to it. Below this is the title 'Epidemiological Situation Report'. Underneath the title is a table with three columns: 'Initiating Event', 'Report Type Type', and 'Date'. The 'Initiating Event' cell contains 'Choose an item.'. The 'Report Type Type' cell contains a drop-down menu with 'Choose an item.' selected and a red circle around the dropdown arrow. The 'Date' cell is empty.

Initiating Event	Report Type Type	Date
Choose an item.	Choose an item.	

Figure 2.



Item Instructions

- 1) Select Program Disease using the drop-down menu.
- 2) Select the initiating event for this report using the drop down menu.
- 3) Select the report type using the drop down menu.
- 4) Enter the date the report is being generated on.
- 5) If this is an affected herd report enter the date the herd was determined to be affected.
- 6) Enter the location of the report herd by State, county, town and zip code.
- 7) Enter the status of the area.
- 8) Select the herd type using the drop down menu.
- 9) Enter the number of animals in the herd by age category.

Narratives:

*NOTE: When submitting updates to previous reports do not delete any previous information! Add new information after previous descriptions and identify it as new information. Methods of identifying new information may be different color type face, **blue recommended**, or highlighting the new text.*

Brucellosis and Bovine Tuberculosis Program Standards

10) Briefly describe the current situation and highlight events or findings that have occurred since the previous report. *Do not delete any previous information!*

Priority Issues

11) Briefly describe any critical actions to occur in the immediate future. Provide dates, if possible.

12) Briefly describe and critical decisions that need to be made and the responsible parties. Provide deadlines, if possible.

13) Briefly describe any accomplishments achieved during reporting period.

14) Briefly describe any planned future activities:

Investigation Summary:

15) Select if the source of the infection was identified using the drop-down menu.

16) Select the source of infection using the drop-down menu.

17) If “other” is selected please describe.

18) Briefly describe any additional report herd details that are considered important but not covered elsewhere. Example: Multiple premises involved.

19) Briefly describe any other information that is believed to be important to the investigation.

Herd Summary:

20) Select the description of the herd type summary from the dropdown menu.

21) Enter the report herd identifier, such as a herd ID number, premises ID number, etc. This should be a unique herd identifier that will be maintained throughout the epidemiological investigation. DO NOT USE THE HERD OWNER’S NAME.

22) For tests conducted in the report herd enter dates, number tested, number suspect, number reactors, number necropsied and any brief comments necessary.

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23) Select if wildlife surveillance is planned, in process, completed, or not planned, or to be determined, using the drop-down menu.

24) Briefly describe surveillance planned or completed. If no wildlife surveillance is planned, describe why.

Within State Epidemiologically Related Herds Summary:

For each epidemiologically related herd within the State initiating the report;

25) Enter the herd identifier. (See 18 above.)

26) Enter the date the herd was tested or is scheduled to be tested

27) Select the reason for testing the herd using the drop-down menu.

28) Enter the number tested, number suspect, number reactors, number necropsied.

Post Mortem Results:

For each animal necropsied;

29) Enter the herd identifier. (See 18 above.)

30) Enter the individual animal official identification.

31) Enter the date the animal was necropsied.

32) Select the gross necropsy results using the drop-down menu.

33) Select the histopathology results using the drop-down menu.

34) Select the PCR results using the drop-down menu.

35) If applicable enter PCR results that identify an agent other than a program disease, i.e. *M. avium*.

36) Select the culture results using the drop-down menu.

Out of State Trace Summary:

37) Enter the State which the trace was sent to.

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- 38) Enter the date the trace documentation was transmitted to the receiving State.
- 39) Select the type of trace being transmitted using the drop down menu.
- 40) Enter a brief description of the action taken by the receiving State as a result of receiving the trace.
Receiving State epidemiologist designated by the District Director to notify originating State o epidemiologist designated by the District Director of actions taken.

Note: Upon receipt of a trace the receiving State should initiate ita own Situation Report describing the handling of the trace within their State.

Appendix 8: Amendment to Animal Health Plan for Recognition of a Management Area

State/Tribe: *Select State from a pull down listing all States and select Tribes.*

Disease of Concern: *Select from a pull down listing Brucellosis and TB*

Category 1. Geographical description

1. Describe the continuous and uninterrupted boundaries of the management area. Additionally, a map may be attached.

Field to enter free text.

Ability to attach PDF or include URL of coverage map

Category 2. Determination of Boundaries and Extent of Infection

2. Complete the table to describe how each of the following activities was used to determine the boundaries for the proposed management area and the extent of the infection in animals within the proposed management area.

Activity	Dates	Brief Description	URL/PDF attachment
Epidemiological investigations (including human and wildlife components, if any).	<i><u>Free text</u></i>	<i><u>Free Text;</u></i>	<i><u>Ability to attach PDF or include URL</u></i>
Surveillance activities within the management area to determine or further delineate sources of disease.			
Surveillance activities outside the boundaries of the management area.			

Brucellosis and Bovine Tuberculosis Program Standards

3. Complete the table to describe how each of the following activities will be used over time to ensure that the boundaries of the management area remain up-to-date.

Proposed Activity	Proposed Timeline	Brief Description	URL/PDF attachment
Epidemiological investigations (including human and wildlife components, if any).	<i>Free text</i>	<i>Free Text;</i>	<i>Ability to attach PDF or include URL</i>
Surveillance within the management area to determine or further delineate sources of disease.			
Program animal surveillance and monitoring activities within the proposed management area, including annual testing of herds and change of ownership/market testing of program animals.		NOTE: This description should include your approach for conducting the required annual testing of herds and change of ownership/market testing of program animals. Include a goal for detection level, sample size goal, and sample selection process in your description.	
Surveillance activities outside the boundaries of the management area.		NOTE: These activities must be sufficient to detect infection in program animals that originate from or are related to the management area.	

Category 3. Sources of disease and assessment of disease spread

4. Complete the following table to describe the approximate number of program animals within and surrounding the proposed management area.

Brucellosis and Bovine Tuberculosis Program Standards

Within the Proposed Management Area		In the Area Surrounding the Proposed Management Area			Source of Estimate
	Number of animals	Number of herds	Number of animals	Number of herds	
Cattle					
Dairy cattle	<u>Number</u>	<u>Number</u>	<u>Number</u>	<u>Number</u>	<i>Pull down: NASS or State census or survey or State animal health Records or Estimated</i>
Beef cattle					
Mixed use					
Bison					
Domestic Bison					
Captive Cervids					
Captive Cervids					

5. Complete the following table to describe the approximate number of susceptible wildlife populations (e.g., tens, hundreds, thousands, etc.) within and surrounding the proposed management area.

Wildlife Population	Approximate number of animals within the management area	Approximate number of animals in area surrounding the management area	Source of Estimate
<u>Free Text</u>	<u>Number</u>	<u>Number</u>	<i>Pull down: NASS or State census or survey or State wildlife Records or Estimated</i>

Ability for user to add rows to table as needed.

6. Complete the following table to describe the number of affected herds or wildlife populations detected in the proposed management area and the prevalence of infection within these affected herds.

Brucellosis and Bovine Tuberculosis Program Standards

	Number of animals tested	Number of infected animals	Animal-Level Prevalence Rate	Number of herds tested	Number of affected herds detected	Herd - Level Prevalence
Cattle						
Dairy cattle	<u>Number</u>	<u>Number</u>		<u>Number</u>	<u>Number</u>	
Beef cattle						
Mixed use						
Bison						
Domestic Bison						
Captive Cervids						
Captive Cervids						
Wildlife						
<u>Free Text</u>						

7. Summarize the likelihood of transmission of the disease of concern from affected herds or wildlife populations to program animals within and surrounding the proposed management area. Include a description of:
 - a. The potential for exposure of program animals to known affected herds or wildlife populations.
 - b. Factors that may influence this potential for exposure (i.e., the movement patterns of animals, seasonal variations in exposures, etc.). {NOTE: Do not list mitigations that your State or Tribe would implement here. These will be described in Category 4.}
 - c. If available, cite or attach a summary of any formal risk assessments concerning the risk of spread of the disease of concern within or from the proposed management area. Otherwise provide a brief qualitative or quantitative assessment of the likelihood of transmission from known affected herd or wildlife populations to program animals within and surrounding the management area.

Field to enter free text.

Ability to attach PDF or include URL of coverage map

Category 4. Risk mitigation measures

8. Describe restrictions that will be placed on the movement of program animals from the proposed management area. Include the timeline for implementation and the plan to monitor and enforce these measures in your description.

Field to enter free text.

9. Describe any other measures that will be implemented to mitigate the risk of disease transmission within and from the proposed management area. Include the timeline for implementation, and the plan to monitor and enforce these measures in your description.

Field to enter free text.

10. If the management area is for brucellosis, attach the brucellosis vaccination program that will be conducted in the management area.

Category 5. Laws and Regulations

11. Briefly describe the statute or regulation that explicitly authorizes the State to establish management areas. In the absence of a statute or regulation explicitly authorizing the State to establish management areas, describe the statute or regulation under which the State claims authority to establish a management area. Include the formal citation, common name, and a Web address for or attach a pdf of the text of the statute or regulation.

Field to enter free text.

Ability to attach PDF or include URL

12. Briefly describe the statute or regulation authorizing the mitigation measures the State has taken or plans to take within the management area. Include the formal citation, common name and a Web address for or attach a pdf of the text of the statute or regulation.

Field to enter free text.

Ability to attach PDF or include URL

Category 6. Personnel

13. Complete the following table to identify the person responsible for implementation and performance of activities and maintenance and enforcement of measures associated with the proposed management area under this animal health plan.

Name	Title	Affiliation	Phone Number(s)	Email
Management Area Responsible Person				
<u>Free text</u>	<u>Free text</u>	<u>Free text</u>	<u>Numbers formatted as (XXX)-XXX-XXXX</u>	<u>Free Text;</u>

14. Complete the following table to identify all personnel assigned to implementation or performance of activities or maintenance or enforcement of measures associated with the proposed management area under this animal health plan.

Name	Role	Affiliation	Phone Number(s)	Email
Animal Health Activities				
<u>Free text</u>	<u>Pull Down:</u> <u>Veterinary Epidemiologist</u> <u>Veterinary Medical Officer</u> <u>Animal Health Technician</u> <u>Animal Identification Coordinator</u>	<u>Pull Down:</u> <u>Federal – APHIS</u> <u>State</u> <u>Tribal</u> <u>Other</u>	<u>Numbers formatted as (XXX)-XXX-XXXX</u>	<u>Free Text;</u>
Wildlife Activities				
	<u>Epidemiologist</u> <u>Veterinary Medical Officer</u> <u>Wildlife Biologist</u>	<u>Pull Down:</u> <u>Federal – APHIS</u> <u>State</u> <u>Tribal</u> <u>Other</u>		

Ability for user to add rows to table as needed.

Category 7. Official Identification

10. Briefly describe the statute or regulation that requires all cattle, bison, and captive cervids that are moved from the management area to be identified with an official eartag prior to movement. Include the formal citation, common name, and a Web address for or attach a pdf of the text of the statute or regulation. In the absence of such a statute or regulation, describe evidence demonstrating that such a requirement will be implemented and enforced.

Field to enter free text.

Designated Brucellosis Epidemiologist

Designated Tuberculosis Epidemiologist

AD

State or Tribal Responsible Person – Management Area

Appendix 9: Performance Standards for Caudal Fold Tuberculin Testing

Each individual authorized to conduct official CFT tests, BTB QAVs, regulatory veterinarians and animal health technicians where applicable, shall be in compliance with these standards for CFT testing if the number of CFT test responders reported is equal to, or greater than, the minimum number of CFT test responders expected for the specified maximum number of CFT tests conducted. For example, if 5 responders have been reported, then the total number of CFT tests conducted should be less than, or equal to, 1050.

The response rates of each individual authorized to conduct official CFT tests shall be calculated for the number of cattle or bison tested for each 1-year period. *Except that:* In the case of authorized individuals who test 300 or less cattle and bison in 1 year, the minimum response rates for the maximum number of CFT tests conducted shall be calculated on a cumulative basis from year to year.

Number of CFT Tests		Expected Minimum Number of CFT Test Responders	Number of CFT Tests		Expected Minimum Number of CFT Test Responders
From	Through		from	through	
0	300	0	3518	3634	26
301	473	1	3635	3750	27
474	630	2	3751	3866	28
631	775	3	3867	3981	29
776	915	4	3982	4095	30
916	1050	5	4096	4209	31
1051	1183	6	4210	4323	32
1184	1315	7	4324	4437	33
1316	1444	8	4438	4551	34
1445	1570	9	4552	4665	35
1571	1695	10	4666	4779	36
1696	1820	11	4780	4893	37
1821	1945	12	4894	5007	38
1946	2070	13	5008	5121	39
2071	2195	14	5122	5235	40
2196	2320	15	5236	5349	41
2321	2445	16	5350	5463	42
2446	2570	17	5464	5577	43
2571	2690	18	5578	5690	44
2691	2810	19	5691	5802	45
2811	2930	20	5803	5914	46
2931	3048	21	5915	6026	47
3049	3166	22	6027	6138	48
3167	3283	23	6139	6250	49
3284	3400	24	6251	6362	50
3401	3517	25	*	*	*

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The table is constructed by assuming that at least 1 percent of non-affected cattle will be false positive on the CFT test. For a given number of responders, the maximum allowable tests are calculated using the cumulative binomial distribution and determining the 5th percentile. For example, 5 or fewer positive test results are expected to occur among less than 5 percent of Accredited Veterinarians who each test, 1050 head of cattle.

* To remain in compliance with these standards, if more than 6,362 CFT tests are conducted prior to the end of 1 year, a minimum of 1 responder must be reported for each additional 110 CFT tests conducted during the remainder of the year.

Appendix 10: Cleaning and Disinfection

The purpose of this appendix is to establish guidelines and minimum criteria for the cleaning and disinfecting of premises determined to be affected by bovine tuberculosis (TB).

Cleaning and disinfecting of all premises, including all structures, holding facilities, conveyances, or other materials which are determined to constitute a health hazard to humans, or animals because of TB is required in Program Standards sections Feedlots and Affected herd management plans.

The following requirements should be adapted as appropriate for feedlots or portions of a feedlot that have contained brucellosis or bovine tuberculosis infected or exposed program animals.

All animals that are identified as affected or exposed must be removed from the premises before beginning the cleaning and disinfecting process. All other animals associated with the affected herd must be removed from the areas to be cleaned and disinfected, and prohibited from re-entering these areas, or commingling with any animals repopulating the premises except as otherwise noted.

Cleaning

All buildings, animal handling, feeding and watering devices, and other equipment as described below, must be power washed with a detergent solution to remove all visible evidence of manure, hay, straw, feed, silage, and other debris.

Buildings

- All building floors, walls, ceilings, and beams must be free of all manure (dried or otherwise), hay, straw, bedding, silage, feed, and other debris.
- Accumulated and dried (“caked”) manure must be scraped off all surfaces.
- Buildings with dirt floors must have all dried manure chunks/piles/pieces removed, all fresh manure shoveled out away from the area being disinfected, and all loose debris swept out.
- Cement floors must be broom-clean.

Animal Handling Equipment

- Pen dividers, corral fencing, alleyways, chutes, etc., must be free of all manure (dried or otherwise), hay, straw, bedding, silage, feed, and other debris.
- Livestock trailers must be free of all manure (dried or otherwise), hay, straw, feed, silage, and other debris.
- All equipment used in the husbandry of the premises must be cleaned of all manure (dried or otherwise), hay, straw, feed, silage, and other debris. This includes, but is not limited to: trucks, tractors, manure spreaders, and alley scrapers. All handling and removal of manure (dried or otherwise), hay, straw, feed,

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silage, and other debris must be completed before performing the cleaning and disinfecting of equipment used for those operations.

Feeding and Watering

- Water troughs, tanks, and tubs must be empty of water and free of all debris.
- Mangers, feed bunks, feed wagons, hay racks, and bale feeders must be free of all manure (dried or otherwise), hay, straw, feed, silage, and other debris.
- Any objects or items left in the area to be disinfected must be soaked in the disinfectant solution. All unused feed, bedding, hay, straw, silage, and salt blocks must be removed beforehand. Remove any items or objects (saddles, clothing, technical instruments, and appliances) that may be damaged by the disinfectant solution.

Disinfecting

- Disinfection must not be performed on any premise that has not completed the pre-cleaning and cleaning processes.
- Disinfection must be performed by, or under, the direct supervision of regulatory personnel. Disinfectant solution must soak into the surfaces and dry naturally.
- Upon completion of disinfection process, regulatory personnel responsible for the disinfection must complete and submit a report of successful completion of the cleaning and disinfection the premises, as required by 9 CFR 50.13.

Manure

- All piled manure, as well as all manure (dried or otherwise), hay, straw, feed, silage, and other debris from pre-cleaning must be spread on fields, not worked into the soil, or may be composted as an alternative to inactivate disease agents. If spreading on fields is the chosen method of inactivation, the detritus must remain on the surface for a period no less than one week of dry weather has elapsed. After the specified time for drying has been met the detritus must be disked into the soil. All susceptible species must be excluded from the field where the detritus is spread until after the material has been disked into the soil.

Appendix 11: Brucellosis or Bovine Tuberculosis Accredited Goat Herds

This appendix provides recommended model procedures to be followed for accrediting and reaccrediting goat herds for brucellosis and bovine tuberculosis (TB).

All goats in an accredited herd must be free from brucellosis and TB. To establish and maintain accredited herd status for brucellosis and TB, the herd owner must comply with all applicable provisions of this document.

The frequency and interval for testing for reaccrediting of goat herds in a particular State or RMA will be the same as accredited herds of program animals.

Testing Requirements and Procedures for Herd Accreditation

Initial accreditation and reaccreditation

- Testing goat herds for accreditation may be conducted only by accredited veterinarians or State/Tribal or Federal animal health veterinarians.
 - To qualify for accredited herd status, the herd must pass at least two consecutive official brucellosis and/or bovine tuberculosis herd tests conducted within an interval of 9-15 months with no evidence of or exposure to brucellosis and TB.
- Additions to the herd during the qualifying period for accreditation: Other than natural additions, any additions to the herd must meet the requirements specified in Accredited Herd Additions
 - When being tested for initial accreditation or reaccreditation, all program species on the premises must have negative brucellosis and TB test results, regardless of whether they are members of the herd.
 - The test observations date (month/year) of the second consecutive herd test of a goat herd that has no evidence of brucellosis and TB will become the anniversary date for herd accreditation.
 - Herds meeting the qualifications for accreditation must be issued a certificate of accreditation, and other appropriate information must be provided by the local State animal health officials to emphasize the significance of the herd accreditation plan.
 - Accreditation periods will be based on the anniversary date of accreditation and not on the dates of reaccreditation testing.
- For herds in consistent States or Tribes, the entire State or Tribe or the portion of a State or Tribe that is not the RMA, if the State or Tribe has an RMA, the accreditation period will be 24 months from the anniversary date.

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- For herds in a provisionally consistent State or Tribe, the entire State or Tribe or the portion of a State or Tribe that is not the RMA, if the State or Tribe has an RMA, the accreditation period will be 12 months from the anniversary date.
- Accredited herds will not be recognized in inconsistent States, Tribes, or RMAs.
- All goats in the herd, including those less than 12 months of age and not nursing, must be officially identified. A report that includes the official identification, age, sex, and breed of each animal and a record of all tuberculin test responses and interpretations must be completed on each herd test for accreditation. Natural additions retained in the herd between herd tests for accreditation or reaccreditation must be identified on the test record as (NA). Purchased additions to the herd between herd tests for accreditation or reaccreditation must be identified on the test record as (PA). Test records must be submitted to the appropriate State and/or Federal animal health officials within 5 business days following the completion of each herd test.
 - To qualify for reaccreditation, the herd must pass a negative reaccreditation herd test within +/- 3 months of the herd's accreditation anniversary date.
 - For continuous herd accreditation, the reaccreditation herd test must be conducted on the anniversary date or within 3 months before the anniversary date.
 - If the reaccreditation herd test is conducted after the anniversary date, the accredited status of the herd will be suspended until the reaccreditation test is completed.
 - If the reaccreditation herd test is not completed within 3 months after the anniversary date, the herd will lose accredited status, and the requirements for reaccrediting the herd will be the same as for initial herd accreditation.
 - Goats included in a herd test for accreditation that respond (demonstrate any visible or palpable response at the site of the tuberculin injection) to the CFT test must be classified suspect.
- Herds containing suspects to the CFT test must be quarantined until the suspect animals are retested negative by the CCT test within 10 days of the CFT injection, or retested negative by the CCT test 60 or more days after the CFT injection. If the CCT test is negative on retest, the herd may be released from quarantine. The CCT test may only be applied by veterinarians employed by the State or Federal government who have received specific training in conducting the test.

Accredited Herd Additions

- Goats other than natural additions added to an accredited herd will not receive the accredited herd status for sale or movement purposes until they have been included in a reaccreditation herd test.
- From herds in a consistent State or Tribe, the entire State/Tribe or the portion of a State/Tribe that is not the RMA, if the State or Tribe has an RMA:
 - From accredited herds: Goats may be moved directly from an accredited herd of origin and immediately commingled with the animals in the destination herd.

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- From herds not accredited: Goats originating from a nonaccredited herd in a consistent State or Tribe must have a negative test for brucellosis and bovine tuberculosis within 10 days of movement. Animals with a negative brucellosis and bovine tuberculosis test may be immediately commingled with the animals in the destination herd.
- From herds in a provisionally consistent State or Tribe, the entire State/Tribe or the portion of a State/Tribe that is not the RMA, if the State or Tribe has an RMA:
 - From accredited herds: Goats originating from an accredited herd in a provisionally consistent State or Tribe must have a negative test for brucellosis and bovine tuberculosis within 10 days prior to movement. Animals with a negative brucellosis and TB test may be immediately commingled with the animals in the destination herd.
 - From herds not accredited: The herd of origin for the additions must have a negative herd test within the previous 12 months, and the individual goats for addition must have a negative brucellosis and TB test within 10 days prior to entering the premises of the accredited herd. Goats may then be immediately commingled with the goats in the destination herd.
- From herds in an inconsistent State or Tribe:
 - No additions to an accredited herd may originate from any herd in an inconsistent State or Tribe.
- From herds in RMAs:
 - Additions that originate from an RMA must reside in a nonaccredited herd outside of the RMA for 4 months, and must meet all relevant testing requirements above, before they are added to an accredited herd.

Herd Inventory Reconciliations

- At each herd test, either initial accreditation or reaccreditation, a complete inventory of all goats in the herd, regardless of age, will be performed.
- All goats present on the current herd test inventory but not present at the previous test inventory must be reconciled as to their origin.
- All goats not present on the current herd test inventory but present at the previous test inventory should be reconciled as to their destination.

Artificial Insemination

Semen used for artificial insemination of goats in an accredited herd must be from sires in accredited herds or from sires with a negative result on an official test for bovine tuberculosis performed within 12 months prior to the date of the semen collection.

Appendix 12: Memorandum of Understanding

(Name of State) Department of Agriculture

and

Animal and Plant Health Inspection Service

Veterinary Services

Memorandum of Understanding

Date

Subject: Agreement for Use of Brucellosis Standard Card Test

To: Director, _____ Region

This memorandum of understanding between the (name of State or Tribe) Department of Agriculture and the Animal and Plant Health Inspection Service establishes agreement on the following policy for the use of the brucellosis standard card test in official brucellosis eradication program work in (name of State or Tribe).

1. The purpose for using the brucellosis standard card test is (indicate intended use and purpose of the brucellosis standard card test [screening and/or confirmatory] for cattle and bison).

Example: Cattle

In field – screening and/or confirmatory

In Veterinary Services (VS)-approved livestock facilities – screening

In laboratory – screening and/or confirmatory

3. The brucellosis standard card test will be performed only by authorized State or Federal brucellosis program personnel or federally accredited veterinarians with an annual authorization issued jointly by the undersigned cooperating animal health officials.

4. The State or Tribal Animal Health Official has specifically designated brucellosis standard card test as the official test for cattle and bison tested at all VS specifically approved stockyards in that State or Tribe.

The following personnel are authorized to perform the brucellosis standard card test:

(List of authorized personnel)

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5. The following regulatory persons are designated to train and administer the annual proficiency tests to qualify to conduct brucellosis standard card tests:

Names: _____

6. Annually, before renewal of the authorization, the authorized State/Tribal and Federal brucellosis program personnel and authorized federally accredited veterinarians must satisfactorily perform the brucellosis standard card test on standardized proficiency test serums using personal equipment (e.g., brucellosis standard card test rocker and cover) at the designated laboratory or at a field site (VS specifically approved stockyard, etc.). A score of 90 percent or above on a minimum of 20 proficiency test samples is required.

In States or Tribes where VS is not funding first point testing (FPT), the National Veterinary Services Laboratories (NVSL) will charge the State, Tribe, or Area for the proficiency test kits. The State or Tribal Animal Health Official and the Associate Director will prescribe which entity (State or Area) will pay for the proficiency test kits.

In States or Tribes where VS is funding FPT, NVSL will provide the proficiency test kits to the State, Tribe, or Area at no charge.

Signed: _____ Date: _____

(State or Tribal Animal Health Official)

Signed: _____ Date: _____

(Associate Director)

Approved

_____ Date: _____

Director, _____ Region, Veterinary Services

Appendix 13: General Instructions and Recommended Procedures for Collecting and Submitting Granulomatous Lesions during Necropsy

Materials and equipment

Sampling and Shipping Supplies

The NVSL will provide the appropriate sampling and shipping supplies upon request. There are several types collection kits available for specific purposes. In general, the supplies needed to sample 1 animal include:

- Two jars (90 mL) of 10% neutral buffered formalin per animal
- Up to 3 Whirl-Paks® (or other sterile bags) per animal for storing fresh tissues
- Two 4 ounce jars of saturated sodium borate solution per animal
- 6 barcodes per animal (2 for the buffered formalin jars (head and chest, abdomen), 2 for the Whirl-Paks® or sodium borate jars, 1 for the datasheet and 1 for the serum vial (if applicable))
- FedEx shipping label

Necropsy Tools

Suggested necropsy supplies include:

- Sharp knife (hunting knives and slaughterhouse boning knives work well)
- Scissors
- Rat-tooth forceps
- Cutting board
- Personal Protective Equipment (see PPE section below)
- Hack saw or bone saw
- Small and large shears (lopping shears or ratcheted rib cutters for rib cage/sternum)
- Chisel and mallet
- Scalpels and razor blades (disposable scalpels are highly recommended)

Other Supplies

Other suggested supplies include:

- Disinfectant
- Scrub brush
- Large rubber tub (for disinfecting boots and necropsy tools)
- Datasheets

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- Plastic bags (large for carcass disposal and small for sample collection)
- Sharpie® or indelible marker (for labeling)
- Pen (for filling out datasheets)
- Plastic sheets, wood chips, pet litter, or other absorbents (for floors in work area)
- Sharps container
- Biohazard waste bag
- Digital camera
- Ruler (for measuring lesions and/or tissue)

Personal Safety Guidelines and Equipment

Bovine tuberculosis is zoonotic and presents a risk to human health and safety. Because of this risk, all carcasses should be handled with caution and considered potentially infectious. Precautions for personal safety should be exercised.

Do not eat, drink, or smoke while dissecting a carcass or collecting samples. Establish a clean work zone and a contaminated work zone (clean/dirty line) with an area to disinfect supplies, equipment, and personnel between the two areas. Place datasheets, camera, and other non-disposable equipment in plastic bags or containers that can be disinfected or discarded.

All personnel conducting necropsies or handling animals that may be infected with bovine tuberculosis should have a bovine tuberculosis test prior to any potential exposure and annually thereafter (or as recommended by an occupational health professional).

Safety and Personal Protective Equipment (PPE)

Wearing protective gear will minimize the possibility of contact with infectious agents in body fluids and aerosols and reduce the risk of human infection. All necropsy tools and instruments should be disinfected before and between necropsies, and after sampling to prevent cross-contamination and infection.

The following PPE are recommended during sample collection:

- Heavy-duty disposable gloves (rubber or nitrile)
- Cut-resistant mesh glove on non-dominant hand
- Goggles, safety glasses, or face shield
- Disposable apron or apron that can be disinfected
- Forearm protectors
- Cloth or Tyvek® coveralls
- Rubber boots
- Hair net or hat that can be disinfected
- Respirator (N95 mask at a minimum)

Work upwind of carcasses when performing necropsies outdoors. Always wash hands and exposed skin with soap and warm water or an alcohol based cleanser after collecting samples.

Handling Harmful Substances

Sodium borate and 10% buffered formalin are hazardous substances that can be inhaled or absorbed through the skin. Carefully handle all harmful substances when sampling and shipping.

Disinfectants are also potentially hazardous and should be handled with care. The Material Safety Data Sheets for each chemical should be reviewed prior to use to ensure that collectors are aware of the dangers associated with handling the disinfectants and chemicals and take the appropriate precautions.

Carcass Disposal and Disinfectants

The carcass and all tissues from the carcass should be disposed of according to State/Tribal and local animal carcass disposal regulations. Depending on the State, Tribe, or area, methods may include burial, incineration, composting or double-bagging and transporting to a landfill. All contaminated paper or plastic materials should be considered hazardous waste and should be thoroughly disinfected, incinerated or double-bagged and disposed of at the landfill (if permitted).

All blood and tissue should be removed from necropsy instruments and tools with soap and water, rinsed, and subsequently disinfected with an approved disinfectant for bovine tuberculosis between necropsies. If performing necropsies in a laboratory setting, a container of 70% ethanol or reagent alcohol with sand in the bottom can be used to decontaminate instruments between animals. The excess liquid should be flamed using a Bunsen burner or propane torch. If disinfectants are not thoroughly rinsed (flamed) from the instrument, a false negative result may occur. If disinfectants are not used between animals, false positives may be identified.

Gloves also should be changed between animals. Necropsy boots, aprons and contaminated clothing should be cleaned and thoroughly disinfected upon completion of sample collections. External surfaces of containers with samples should be disinfected.

The products listed in the table below are effective, environmentally friendly disinfectants for use against bovine tuberculosis. Additional approved bovine tuberculosis disinfectants can be found in the U.S. Environmental Protection Agency Office of Pesticide Programs List B: EPA’s Registered Tuberculocide Products Effective Against *Mycobacterium tuberculosis*.

Disinfectant	Time to Effectively Disinfect	Environmentally Friendly	Manufacturer
Oxivir bovine tuberculosis	5 minutes	Active ingredients break down to water and oxygen	Johnson Diversey
Opti-Cide 3	3 minutes	Contains no dangerous phenols, chlorine, artificial dyes or perfumes	Micro-Scientific Industries
Clorox Bleach (Mix 1 part bleach with 9 parts water)	5 minutes	Product contains no free chlorine and breaks down into salt and water after use; does not contain dioxins or contaminate groundwater	Clorox Company

Disposal of Gloves and Sample-Related Waste

Spray or soak waste with disinfectant and place in bag. Then spray or soak bag with disinfectant, place in another bag, and dispose at the landfill (if permitted).

Collecting Specimens

General Recommendations

Despite the stringent decontamination protocols used in the laboratory, tissue specimens can still be overgrown by environmental fungi and bacteria, thereby impeding the ability to recover any viable mycobacteria present in the tissues. To minimize overgrowth, it is important to collect tissues from the animal as soon as possible post mortem. Collect tissues from animals within 2 hours whenever possible. *If the animal has been dead for more than 24 hours, contact the laboratory prior to submitting samples for histopathology.* When performing the necropsy, collect the tissues using aseptic techniques from the head, thoracic cavity and abdominal cavity (in that order) to minimize cross-contamination.

Sample Collection and Storage

Collect tissues from animals within 2 hours when possible and ship to NVSL within 72 hours. Delays in shipping samples to NVSL may result in false negative results. Extended exposure to sodium borate may make the recovery of *M. bovis* impossible. Extended exposure to 10% buffered formalin will denature the proteins and render the samples unsuitable for testing.

Samples submitted for histopathology should be placed in 10% buffered formalin. Samples submitted for culture should be collected in Whirl-Paks® and kept cold until shipped or in sodium borate at room temperature. Tissues from the head and chest can be combined in the same Whirl-Pak® or jar. However, lymph nodes or tissues from the abdominal region should be placed in separate containers to prevent contamination. Fresh, well preserved samples increase the probability of culturing and detection of *M. bovis*.

Samples to Collect

Collection of Lesions or Abnormal Tissue

- Observed lesion(s) should be submitted in separate containers for laboratory analysis.
 - Do not include lesions in the same containers with regularly collected lymph nodes or tissues, or in a container with other lesioned tissue samples.
 - Label the container (not the lid) with the location where lesions were found.
 - When submitting tissue with lesions, be sure to document the following in the comments section of the datasheet: the origin of the tissue affected, number of lesions, distribution and pattern, size, shape, color, consistency and texture of the lesion.
 - Additionally, submit a digital photograph of all of the lesions with an adjacent object that indicates the scale of the lesion, if possible.
 - When multiple lesions are found in the same tissue, submit a small section (the size of a golf ball) for histopathology and culture and include sections of adjacent normal tissue.
 - If only 1 lesion is found, divide the tissue in half through the middle of the lesion and submit half for histopathology and half for culture (include adjacent normal tissue).
 - If there are insufficient lesions to divide, submit the available tissue for culture testing only.

- Blood should also be collected from animals exhibiting gross lesions suggestive of bovine tuberculosis infection.

Collection of Normal Tissue

Collect submandibular, retropharyngeal, tracheobronchial, mediastinal, and mesenteric lymph nodes from each animal, as well as a section of tonsils, lungs, and any tissues with gross lesions. Carefully examine the lungs and palpitate for abnormalities.

Tissue Selection and Preservation

7. Always clean and disinfect instruments between necropsies of each animal. If disposable scalpels are available, discard them after each animal. Thoroughly rinse instruments after disinfecting to ensure that the samples are not inadvertently disinfected. Change gloves between the necropsy of each animal.
8. Submit lymph nodes in separate containers based on the region of the body from which they are collected (head and chest combined, and abdomen in a separate container). Use 2 formalin jars and 2 Whirl-Paks® if no gross lesions are observed. *Note: Tissues with lesions should be submitted in separate containers for culture and labeled with the tissue type in addition to the barcode.*
9. Label the Whirl-Paks® with the last 4 digits of the barcode and “HC”, or “A” to correspond to the head and chest, or abdomen (see picture below). After the samples have been collected, place all Whirl-Paks® in a larger Ziploc® bag with the barcode affixed to the Ziploc® bag.
10. Assign a unique barcode to each animal. Apply a barcode with the same number to the formalin jars, the Ziploc® bag (with Whirl-Paks® inside), the serum vial, and the datasheet. *Tissues from different animals should never be combined in the same jars or Whirl-Paks®.*
11. For samples submitted for histopathology, divide the tissues or lymph nodes into slices approximately $\frac{3}{8}$ inch thick (width of a pencil). Place lymph nodes or tissues in the formalin jar. If lesions are observed, collect sections of the lesions and include normal tissues surrounding the lesions. Lymph nodes submitted for histopathology should be cut in half to ensure that they become formalin-fixed (unless they are the size of a jelly bean or smaller).
12. For small lymph nodes, do not cut lymph nodes in half when submitting for culture. Store fresh tissues in Whirl-Paks® and then place the samples on ice. For larger ($>\frac{3}{8}$ inch thick) lymph nodes, cut $\frac{3}{8}$ inch sections for formalin and submit the remainder for culture. For collection of samples using sodium borate, cut a 1 inch cube of tissue (approximately the size of a golf ball) and place the lymph node or tissue into the sodium borate jar. *Tissues less than 1 inch thick should not be placed in sodium borate.*
13. If the sample volume is insufficient to divide for histopathology and culture, it is recommended that samples are submitted for culture.
14. After sample collection, disinfect the outside of each Whirl-Pak® or container in an approved disinfectant for bovine tuberculosis. Use caution to ensure that no disinfectant enters the Whirl-Pak® or the container. Remember to keep the surface wet with disinfectant solution for the required contact time (see the table on page 13 or refer to the product label). Rinse with water after the contact time requirement has been met.
15. Tighten the caps on specimen containers and seal with Parafilm®. Electrical tape also can be used if Parafilm® is not available. Place a barcode on the outside of the specimen container. The Whirl-Paks® for culture and the formalin jars for histopathology will receive the same number because they are from the same animal.

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16. Do not freeze specimens that will be submitted for histopathology. Fresh tissues for culture submitted in Whirl-Paks® should be kept cool on ice or refrigerated until shipped to the laboratory. If fresh tissues will be held more than 3 days before shipping, freeze at -20°C and ship frozen tissues on ice packs. Formalin fixed tissues as well as fresh tissues stored in sodium borate can be kept at room temperature until shipped.
17. Samples should be shipped to the NVSL within 24 hours of collection when possible. Avoid weekend delivery unless prior arrangements have been made with the laboratory staff (See shipping section below for instructions and address).

Blood Collection

If bovine tuberculosis or bovine tuberculosis-like granulomas or lesions are observed anywhere in the carcass, use a needle and syringe to extract as much blood as possible from the heart.

1. Place extracted blood into a 10 mL red-top or serum-separator tube (collect at least 2 tubes if possible).
2. Blood should be left undisturbed for approximately 30 minutes at room temperature to encourage clot formation prior to centrifugation.
3. Centrifuge for 10 minutes at a minimum of 1,800 revolutions per minute for 15 minutes to separate the serum from the blood cells. Then use a sterile disposable pipette to transfer the serum to a polypropylene Cryovial® (or pour serum off if using a serum-separator tube). If a centrifuge is not available, serum can be obtained by letting the clot or blood cells settle, then chill in refrigerator to contract the clot, and then transfer the serum to a polypropylene Cryovial®.
4. Submit at least 1 mL of serum (more if possible) to the bovine tuberculosis serum bank. Transfer the serum to a single polypropylene Cryovial® and refrigerate, or transfer 0.5 mL of serum into 1.2 mL polypropylene Cryovials® and freeze at -20°C.
5. Label the serum vial with the sample barcode number using a Sharpie® or permanent marker.
6. Refrigerated serum should be shipped to NVSL with ice packs within 2 weeks of collection.
7. Frozen serum can be shipped to NVSL once a month on dry ice to ensure the samples remain frozen during shipping. *Do not freeze serum samples that contain more than 0.5 mL of serum.* This will reduce the number of freeze-thaw cycles during processing at NVSL to ensure that sample quality is maintained.

Submit all serum to:

National Veterinary Services Laboratories
Attention: Jeff Nelson, bovine tuberculosis Serum Bank
1920 Dayton Avenue
Ames, Iowa 50010

Questions regarding serum collection or handling procedures should be directed to Jeff Nelson by email at Jeffrey.T.Nelson@aphis.usda.gov or by telephone at (515) 337-7966.

Appendix 14: VS Form 10-7

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 0579-00xx. The time required to complete this information collection is estimated to average xx 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
0579-00xx

UNITED STATES DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE NATIONAL VETERINARY SERVICES LABORATORIES P.O. BOX 844, 1920 DAYTON AVENUE, AMES, IA 50010 (515) 337-7514	SPECIMEN COLLECTION- Bovine Tuberculosis Reactors, Suspects, and Trace-Exposed	Use this form only as a supplement to VS Form 10-4. See reverse for instructions.
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1. SUBMITTER NAME (including Business Name)	2. NVSL SUBMITTER ID	3. NAME OF OWNER (no owner)	<input type="checkbox"/> Check if wildlife
---	----------------------	-----------------------------	--

4. IDENTIFICATION

Official Animal ID	Herd/Management Tag	Breed	Age	Sex
TRACE-EXPOSED ANIMAL?	YES	NO	FROM WHICH AFFECTED HERD (NAME OR STATE)	

5. PRIOR TEST RESULTS (CHECK APPROPRIATE BOX)

CAUDAL FOLD (CFT) TEST	POSITIVE	NEGATIVE	NOT DONE
COMPARATIVE CERVICAL (CCT)	REACTOR	SUSPECT	NOT DONE
INTERFERON GAMMA ELISA RESULT	NOT DONE		
GROSS LESIONS AT NECROPSY	YES	NO	

6. EXAMINE and SUBMIT the following LYMPH NODES (LN):

SAMPLE ID	LN	LESION	COMMENTS
	HEAD LN POOL		
	MEDIAL RETROPHARYNGEAL	YES	NO
	LATERAL RETROPHARYNGEAL	YES	NO
	MANDIBULAR	YES	NO
	PAROTID	YES	NO
	THORACIC LN POOL		
	TRACHEOBRONCHIAL	YES	NO
	CRANIAL, MIDDLE, CAUDAL MEDIASTINAL	YES	NO
	ABDOMINAL LN POOL		
	MESENTERIC	YES	NO
	HEPATIC	YES	NO

**7. EXAMINE the following tissues but submit ONLY if lesioned:
Lung, pleura, liver, spleen, ovaries, uterus, prescapular LN, cervical LN, popliteal LN, mammary LN, iliac LN**

SAMPLE ID	BRIEF DESCRIPTION OF LESIONED TISSUE

VS FORM 10-7 INSTRUCTIONS

THIS FORM IS INTENDED AS A SUPPLEMENT TO VS FORM 10-4 AND MUST BE ACCOMPANIED BY VS FORM 10-4. ALL information must be printed legibly or typed. **USE A SEPARATE FORM FOR EACH ANIMAL.**

1 and 2. SUBMITTER CONTACT INFORMATION

Enter the submitter's business name/affiliation and NVSL Submitter ID (if available) exactly as entered on VS Form 10-4.

3. OWNER INFORMATION

Enter the name of the animal owner as entered on VS Form 10-4.

4. IDENTIFICATION

Sample ID – Ensure the sample identification number on this form matches the sample identification number placed on the specimen container.

Official Animal ID – Record the animal's national identification tag number. NOTE: Laboratory results will be reported by animal identification number.

Herd/Management Tag – Record the identification used within the herd or management system.

Breed – Enter the animal breed (e.g., *Holstein*, *Angus*).

Age – Indicate the approximate age in years (*y*) or months (*m*)

Sex – Indicate the sex, male (*M*), or female (*F*).

5. PRIOR TEST RESULTS

Enter the results of prior tests and examinations performed on the animal.

6. EXAMINE AND SUBMIT LYMPH NODES

Examine and submit the indicated lymph nodes. Check whether lesions were noted on each tissue and add any pertinent comments.

Unless otherwise directed by a USDA tuberculosis epidemiologist, use separate containers for head, thoracic and abdominal lymph nodes from the animal, including those with no gross lesions.

Ensure the sample identification number on this form matches the sample identification number placed on the specimen container.

7. EXAMINE OTHER TISSUES. SUBMIT ONLY IF LESIONS ARE FOUND.

Examine each tissue listed in this section but submit samples only if lesions are found. Submit lesioned tissues in separate containers from lymph nodes listed in Block 6. Provide a brief description of the lesions found on each submitted tissue.

The FSIS Guide book may be helpful for identifying tuberculosis lesions:

www.aphis.usda.gov/animal_health/animal_diseases/tuberculosis/downloads/tb_guidebook.pdf for instructions on submitting tuberculosis lesions and/or thoracic granulomas.

I. TISSUE SELECTION AND PRESERVATION

- A. AVOID CONTAMINATION – Remove excess fat.
- B. Divide lesions in half. Place one portion in formalin for histopathology and place the remaining portion in borate or whirl-pack for culture.
- C. HISTOLOGY PORTION: Cut specimen, including normal tissue surrounding lesion, into slices approximately 1 cm (½ inch) thick. Prior to placing in formalin.
- D. CULTURE PORTION: Place the intact portion of the sample into borate or whirl-pack. Do NOT cut the sample into slices.
- E. Maximum tissue to preservative ration: Formalin – 1:10
Borate – 1:1

II. IDENTIFYING DEVICES

If the identifying devices will not be held locally, place the identifying devices from each animal in a plastic bag, and send to the NVSL in the box with the specimens.

III. SHIPPING SAMPLES

- A. Shipping containers are available from the National Veterinary Services Laboratories. Contact the shipping department at 515-337-7530 or NCAH.Shipping@aphis.usda.gov
- B. No refrigeration is required for borate or formalin. Ice packs are required for fresh tissue. DO NOT FREEZE; freezing ruins specimens
- C. PREVENT LEAKAGE – Tighten and tape caps.
- D. SECONDARY CONTAINER – Place samples in a leak-proof bag
- E. ABSORBENT PAD – Place absorbent material in bag with samples to absorb any leakage.
- F. IDENTIFYING DEVICES – Place in separate plastic bag with samples.
- G. SHIPPING CONTAINER- Insert sealed secondary container into an approved diagnostic shipping container and seal.
- H. SUBMISSION FORMS – Place between sealed secondary container and outside mailer.
- I. RETURN ADDRESS – Provide complete return address on mailing label.

Ship submissions to:

USDA, APHIS
NATIONAL VETERINARY SERVICES
LABORATORIES
1920 DAYTON AVE
AMES, IOWA 50010
TELEPHONE NUMBER (515) 337-7212

IV. ADDITIONAL GUIDANCE

For questions regarding histology, contact the NVSL Pathobiology Laboratory at 515-337-7912.

For questions regarding bacteriology, contact the NVSL Diagnostic Bacteriology Laboratory at 515-337-7388.

Appendix 15: Specific instructions for completing VS Form 1-27, Permit for Movement of Restricted Animals

Items 1, 2, and 3 – Self-explanatory.

Item 4 – Name and address of the person who owned the animal(s) at the time the infection or exposure status was determined.

Items 5, 6, 7, and 8 – Self-explanatory.

Item 9 – Show the number of known reactors and exposed animals.

Item 10 – Indicate whether the status of the herd of origin of the animals was affected, exposed, or suspect.

Item 11 – List the status of the area as it applies to the disease condition listed in item 8.

Items 12, 13, and 14 – Self-explanatory.

Item 15 – Record seal number when a seal is used in accordance with program instructions.

Item 16 – Vehicles moving tuberculosis or reactor animals interstate, in accordance with applicable APHIS regulations, must be cleaned and disinfected at destination.

Item 17 – Show the eartag numbers of each animal in the “Complete Ear Tag No.” column, with the identification number being recorded above the reactor tag number. List the breed, sex, and disease brand (T or S) of each animal in the appropriate columns. The “Other Identification” column is used to show sale, bangle, and back tag numbers; identification brands; and any distinguishing marks on each animal. The term “TB tattoo” is recorded in this column if owners exercise the option of permanently identifying their reactors by tattooing the letters “TB” in the reactor’s left ear (rather than branding them with a “T” on their hip).

Item 18 – The signature of the person responsible for performing the inspection (State, Tribal, Federal, or accredited veterinarian; Federal animal health technician; or State or Tribal animal health official).

Items 19 and 20 – Self-explanatory.

Items 21 and 22 – Allow a reasonable time for the movement to take place. Permission to move the animal is void after the date and time shown here.

Item 23 – The signature of the owner or shipper. If the owner or shipper is not available, the trucker may sign. This should never be signed by the inspector or the market organization unless the market is the buyer/shipper.

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Item 24 – Check the applicable block or, if the trucker signed Item 23, write “trucker” in the available space of this block.

Items 25 through 30 – Self-explanatory.

Item 31 – Signature of person breaking the seal, when applicable.

Item 32 – Self-explanatory.

Items 33 and 34 – When the inspector at the slaughter plant cannot certify through personal knowledge as to receipt and slaughter, and if the plant management satisfies the inspector that the animals have, in fact, been handled properly, the inspector can insert in the space above Item 28, “Plant Records” or “Plant Management” and then sign Item 33 and date Item 34.

Appendix 16: Brucellosis Standard Card Test Authorization

(Name of State or Tribal) Department of Agriculture

and

Animal and Plant Health Inspection Service

Veterinary Services

Brucellosis Standard Card Test Authorization

Authorization No.: _____

Date Issued: _____

Expiration Date: _____

Authorized User's Name: _____

Authorized User's Address: _____

You are authorized to perform the brucellosis standard card test only for official brucellosis program testing in the State or Tribe of (name of State or Tribe).

Conditions for performing the test:

1. For each brucellosis standard card test, follow the directions included in the brucellosis standard card test kit.
2. Promptly submit all blood samples with properly completed test records to a Veterinary Services (VS)-approved brucellosis diagnostic laboratory for retesting. Ensure that samples are of sufficient quality and quantity to permit retesting.
3. Federally accredited veterinarians are authorized to conduct tests only at the office or at the VS specifically approved stockyard. State/Tribal and Federal personnel are authorized to conduct the test at locations and for purposes approved by the Associate Director (AD).
4. Retain proficiency to accurately diagnose positive and negative sera. VS will determine proficiency by continually reviewing brucellosis confirmatory tests and retest results at VS-approved brucellosis diagnostic laboratories.

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5. Before annual renewal of the authorization, satisfactorily conduct the brucellosis standard card test on standard proficiency test serums using personal equipment (e.g., brucellosis standard card test rocker and cover) at the designated laboratory or at a field site (VS specifically approved stockyard, etc.). You must score 90 percent or above on a minimum of 20 proficiency test samples.
6. Complete a brucellosis standard card test notice acknowledging receipt of brucellosis standard card test kits and antigens issued and prevent the unauthorized use of the test kits and antigen.

The Animal and Plant Health Inspection Service may suspend or revoke your Federal accreditation if you do not meet these conditions.

ENDORSED _____ Date: _____
(Authorized User)

ENDORSED _____ Date: _____
(Standard Card Test Training Officer)

APPROVED _____ Date: _____
(State or Tribal Animal Health Official)

APPROVED _____ Date: _____
(Associate Director)

Appendix 17: Brucellosis Standard Card Test Notice

1. Authorized user: Please read and acknowledge by signing below and return to the Associate Director (AD).

Brucellosis Standard Card Test Notice

This brucellosis standard card test kit and antigen for animal brucellosis testing are the property of the U.S. Department of Agriculture. The U.S. Government is providing these materials for your use when performing official tests for brucellosis under the Cooperative State-Federal Brucellosis Eradication Program only. Do not use these materials for any purposes other than those for which you are authorized. When no longer needed or authorized, return the kits and antigen to the issuing official.

Converting this kit or antigen or using this kit and antigen for your personal use or for the use of another is theft and embezzlement of Government property and is a violation of title 18, section 641, of the United States Code.

Any theft or embezzlement of Government property is punishable by criminal fines, imprisonment, or both. In addition, the Animal and Plant Health Inspection Service will suspend or revoke your accreditation, if you are a federally accredited veterinarian.

This is to certify that I have received, read, and understand this notice.

Authorized User's Signature

Official Title

Print Authorized User's Name

Date: _____

2. AD: Please check the applicable box below, complete the name and shipping address of the authorized user, sign, and submit to the National Veterinary Services Laboratories (NVSL).

___ NVSL **will charge** authorized user – APHIS is not funding First Point Testing in the authorized user's State or Tribe.

___ NVSL **will not charge** authorized user – APHIS is funding First Point Testing in the authorized user's State or Tribe.

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Name and Shipping Address of Authorized User:

Name: _____

Address: _____

Signature: _____

Associate Director

Date: _____

3. NVSL: Complete the brucellosis standard card test information below. After sending the kit(s) and/or antigen to the authorized federally accredited veterinarian or authorized State, Tribal, or Federal personnel, **return a copy of this notice to the AD.**

Kit Serial No. (150-KIT): _____

Antigen Serial No. (150-AMP): _____

No. of Kits Sent: _____

No. of Kits Sent: _____

Expiration Date: _____

Appendix 18: Protocol for Ordering Brucellosis Standard Card Test Kits, Cards, and Antigen

Submit all orders for brucellosis standard card test kits, cards, and antigen to the National Veterinary Services Laboratories (NVSL), Ames, Iowa.

In States or Tribes where the Animal and Plant Health Inspection Service (APHIS) is funding first point testing (FPT):

Authorized brucellosis standard card test users may continue to order, at no charge, brucellosis standard card test kits, cards, and antigen from NVSL. **Federally accredited veterinarians are authorized to use the brucellosis standard card test for FPT only. The authorized federally accredited veterinarians or authorized State, Tribal, or Federal personnel must receive approval (brucellosis standard card test notice) from the Associate Director (AD) before ordering brucellosis standard card test kits, cards, or antigen.** A brucellosis standard card test notice must be completed and approved by the AD before the purchase and shipment of the brucellosis standard card test kits and antigen from NVSL to verify that the order is approved. The AD will fax a copy of the brucellosis standard card test notice to NVSL to verify that the order is approved. The AD will also indicate on the brucellosis standard card test notice that the order is **not** to be charged for and provide the shipping address. The AD will give NVSL a list of persons **authorized** to receive the kits and antigen.

VS-approved brucellosis diagnostic laboratories in States or Tribes where APHIS is funding FPT may continue to order, at no charge, brucellosis standard test kits and brucellosis standard card test antigen from NVSL for testing blood samples from FPT sites and slaughter facility sites in those States. **The laboratory may charge a fee to test blood samples from FPT sites in States where APHIS is not funding FPT.**

Veterinary Services (VS)-approved brucellosis diagnostic laboratories will not order brucellosis standard card test kits or antigen to supply other VS-approved brucellosis diagnostic laboratories or authorized users for FPT.

In States or Tribes where APHIS is not funding FPT:

Authorized brucellosis standard card test users may order brucellosis standard card test kits, cards, and antigen from NVSL. **A fee will be charged to the authorized user for the kits and antigen.** The authorized user will enter into a user fee agreement with NVSL to facilitate billing. **Federally accredited veterinarians are authorized to use the brucellosis standard card test**

for FPT only. The authorized users must receive approval (brucellosis standard card test notice) from the AD before ordering brucellosis standard card test kits, cards, or antigen. The AD will complete and approve a **brucellosis standard card test notice** before the purchase and shipment of the brucellosis standard card test kits and antigen from NVSL. The AD will fax a copy of the brucellosis standard card test notice to NVSL to verify that the order is approved. The AD will also indicate on the brucellosis standard card test notice to whom the order is to be charged and provide the shipping address. The AD will give NVSL a list of persons **authorized** to receive the kits and antigen.

VS-approved brucellosis diagnostic laboratories receiving FPT blood samples from FPT sites in States or Tribes where VS is **not** funding FPT may continue to order brucellosis standard card test kits and antigen from NVSL. **The laboratories will be charged for kits and antigen used to test cattle and bison sera from States or Tribes where APHIS is not funding FPT.** NVSL will charge for proficiency test kits provided to laboratories in States or Tribes where APHIS is not funding FPT. The laboratory will enter into a user fee agreement with NVSL to facilitate billing. **The laboratory may charge a fee to test blood samples from FPT sites in States or Tribes where VS is not funding FPT.**

VS-approved brucellosis diagnostic laboratories will not order brucellosis standard card test kits and antigen to supply other VS-approved brucellosis diagnostic laboratories or for authorized users for FPT.

Contact Information

National Veterinary Services Laboratories
Brucella and Mycobacterium Reagents Team
1920 Dayton Avenue
Ames, IA 50010
Phone: 515-337-7181
Fax: 515-337-7284

VS-Approved Brucellosis Diagnostic Laboratories

The current VS-approved brucellosis diagnostic laboratories list is available at

http://www.aphis.usda.gov/animal_health/lab_info_services/downloads/ApprovedLabs_Brucella.pdf

Appendix 19: Suggested Template for Owner Participation Projects to Evaluate New TB Tests

Owner: _____

Address: _____

I agree that my participation is voluntary.

I agree to test my animals using new tests under evaluation. I understand that these tests are not yet approved for official TB program use.

I agree that my animals will also be tested using official TB tests. For cattle and bison, official TB tests are the caudal fold tuberculin skin test, the comparative cervical tuberculin skin test, and the interferon gamma blood test. For captive cervids, official TB tests are the single cervical tuberculin skin test and comparative cervical tuberculin skin test, and, for elk, red deer, white-tailed deer, fallow deer, and reindeer, the Stat-Pak[®] and DPP[®] tests.

I agree that any animals that have a non-negative result to the test under evaluation may be euthanized by VS for further diagnostic testing, even in cases where the official TB tests are negative. VS will provide indemnity for such animals through the indemnity process. If indemnity funds are not available for non-negative animals, I agree to cooperate with State, Tribal, and Federal officials to have follow-up live animal TB testing performed on these animals.

I agree to notify VS if an animal is sent to slaughter or dies, so that specimens may be collected and submitted for diagnostic testing.

I agree that if TB is confirmed in an animal by approved laboratory tests, the herd will be considered TB-affected and managed according to TB program requirements.

I agree that other than indemnity payments for animals with non-negative test results, VS will not compensate me for participation.

Brucellosis and Bovine Tuberculosis Program Standards

I agree that animals that have a non-negative response to official TB tests will be managed according to TB program requirements, regardless of the result for the test under evaluation.

Owner signature and date

Witness signature and date

