

Comparative Cervical Test (CCT) for Bovine Tuberculosis

Purpose - The purpose of this standard operating procedure is to provide instruction for conducting a comparative cervical tuberculosis (CCT) test.

Background – Tuberculosis (TB) is a contagious disease of both animals and humans. It is caused by three specific types of bacteria that are part of the Mycobacterium group: Mycobacterium bovis, M. avium, and M. tuberculosis. Bovine TB, caused by M. bovis, can be transmitted from livestock to humans and other animals. Cooperative Federal-State-Industry efforts seek to eradicate bovine TB from cattle and cervids in the United States. Wisconsin currently maintains a Free status in the Bovine TB eradication program and a Modified Accredited status in the Cervid TB eradication program. The Division of Animal Health issues quarantines, and in cooperation with USDA, APHIS, Veterinary Services performs epidemiology, testing, and other bovine tuberculosis control and eradication activities.

Scope – This SOP applies to regulatory veterinarians who are responsible for conducting comparative cervical tuberculosis testing.

Definitions –

“BFS” means the Bureau of Field Services, within the Division of Animal Health, Wisconsin Department of Agriculture, Trade and Consumer Protection

“CFR” means Code of Federal Regulations

“Comparative Cervical Tuberculosis Test” – defined by the UM&R as: “The intradermal injection of biologically balanced USDA bovine PPD tuberculin (CCT) test tuberculin (0.1 ml) and avian PPD tuberculin (0.1 ml) at separate sites in the midcervical area to determine the probable presence of bovine tuberculosis (M. bovis) by comparing the responses to the two tuberculins at 72 hours (plus or minus 6 hours) following injection. The CCT injection must occur either: (1) within 10 days following the CFT injection or (2) more than 60 days following the CFT injection. This test shall be administered only by a State or Federal veterinarian specifically trained in the application of the test.”

“Department” and “DATCP” means the Wisconsin Department of Agriculture, Trade and Consumer Protection

“Division” and “DAH” mean the Division of Animal Health within the Wisconsin Department of Agriculture, Trade and Consumer Protection

“Field Veterinarian” means a DATCP, DAH District Veterinarian or a USDA, APHIS, Veterinary Services Veterinary Medical Officer assigned to a district within Wisconsin

“Program Manager” means the Wisconsin designated tuberculosis epidemiologist or the person responsible for directing the cooperative bovine tuberculosis control program in Wisconsin

“Suspect” or “Responder” means any animal that shows a reaction to the caudal fold tuberculosis test (bovine) or single cervical test (cervid)

“Tuberculosis” or “TB” refers to bovine tuberculosis, caused by Mycobacterium bovis

“UM&R” means Bovine Tuberculosis Eradication Uniform Methods and Rules (for bovines use January 1, 2005 edition, for cervids use January 22, 1999 edition)

Responsibilities –

Regulatory veterinarians provide confirmatory tuberculosis skin testing by using the CCT for animals that have responded to the tuberculosis skin testing performed by private veterinary practitioners. The CCT must be applied within 10 days of the initial test injection. If not, a waiting period of 60 days (bovine) or 90 days (cervid) is required until the CCT can be applied. Field veterinarians also issue and release quarantines and conduct epidemiologic investigations as indicated. Private veterinary practitioners usually report tuberculosis responders directly to the District Veterinarian or federal VMO for their area.

Procedure –

1. The Field Veterinarian either receives notification of a suspect skin test directly from the private veterinary practitioner or the notification is relayed through the designated tuberculosis epidemiologist, Program Manager, or other staff.
 - a. Due to the time-sensitive nature of the CCT, private veterinary practitioners are instructed to make sure they get a reply from the Field Veterinarian when reporting suspects. They should talk directly to the Field Veterinarian, or receive a direct reply to a text or email message. Practitioners should not just leave a message and assume that the TB suspect notification is complete.
 - b. Private practice veterinarians are encouraged to notify the Field Veterinarian prior to scheduling TB testing in herds where they expect to find one or more suspects, so that the Field Veterinarian can reserve time to do the CCT within the 10 day window.
2. The Field Veterinarian responds to the suspect report
 - a. The CCT must be applied within 10 days of the initial test injection. If this testing window is missed, the herd must remain under quarantine and the CCT will be applied after the 60 day (bovine) or 90 day (cervid) waiting period.
 - b. A copy of the 6-22 form or written documentation from the testing veterinarian, which includes the suspect animal/s identification and injection and read dates, must be provided to the regulatory veterinarian. If documentation is not available at the time that the regulatory veterinarian arrives at the farm to test the suspect animal(s), the herd will be quarantined and no testing will be conducted until required documentation is made available to the regulatory veterinarian. If the 10 day window to apply the CCT following CFT is missed, the herd will remain under quarantine until animals are eligible to be tested (more than 60 days after CFT injection for bovines, more than 90 days for cervids.)
 - i. In lieu of the 6-22 Tuberculosis Test Report form, written documentation from the veterinary practitioner, such as an email or text message from the testing veterinarian, is acceptable if it includes:
 1. The date of the CFT or SCT injection

2. The official identification, ancillary identification, age, sex, and breed of the responders
 3. Owner/manager contact information
 4. Animal location
 - c. The Field Veterinarian contacts the herd owner or manager to schedule the CCT and notifies them that the herd is quarantined until the status of the suspect is resolved.
 - d. Education for the owner about tuberculosis, the zoonotic nature of tuberculosis, the reason for the CCT, and the possible outcomes of the CCT (negative, suspect, reactor) may be needed at this time or at the time of the CCT injection.
 - e. It is a good idea to obtain epidemiological information about the potential tuberculosis exposure history of the herd at this time, or at the time of the CCT injection.
3. Quarantine the herd
 - a. Serve the written quarantine at the time of CCT injection, unless circumstances require the quarantine to be served sooner.
 - i. See policies DIS-021 Completing, Issuing, and Serving an Order of Quarantine, and DIS-004 Field Issued Quarantines for Caudal Fold and Single Cervical Tuberculin Responders
 - ii. If there is a delay in applying the CCT beyond 10 days from the initial test, the herd quarantine will be served to the farm, and the quarantine sent to the DAH office. These extended quarantines will be recorded, tracked, and released from the DAH office upon successful resolution of any suspects after the required 60 or 90 day waiting period.
 - b. The quarantine will be written to apply to the entire herd, including animals on other premises owned and/or operated by the same entity, unless there are biosecurity measures in place to prevent commingling
 - c. The terms of the quarantine are provided as standard language on the back of the quarantine form and can be referenced by number in the Terms of Quarantine section. If deviations from the quarantine terms are needed, consult the tuberculosis epidemiologist/Program Manager for the correct wording of the quarantine terms.
4. Apply the CCT – see 9CFR Part 77–Tuberculosis, and also Bovine Tuberculosis Eradication Uniform Methods and Rules January 1, 2005 (for bovines), and January 22, 1999 (for cervids) for further information. See VS Guidance 6705.1 Tuberculin Storage and Handling for proper storage and care of the tuberculin.
 - a. Restrain the animal
 - b. Verify the animal identification and record any ancillary identification not included on the practitioner’s test report
 - c. Verify the tuberculin is a matched pair and record the lot numbers
 - d. Clip two sites on the side of the neck in the mid-cervical region
 - i. #40 (surgical clip) blade
 - ii. avoid any area that already has swelling or irritation that could interfere with reading the test
 - iii. in cervids, use the opposite side of the neck than was used for a SCT

- iv. Note any scars or abnormalities to avoid confusion when reading the reaction
- e. Using appropriate calipers, measure and record the skin thickness of each site before injecting. (measure to the nearest 0.5 mm for bovine, to the nearest 0.1 mm for cervids)
- f. Also record which side of the neck was used (left or right) and any abnormalities noted.
- g. Inject 0.1 ml of PPD avian in one site and 0.1 ml PPD bovis in the other site (see NVAP Reference Guide for Control and Eradication of tuberculosis for more details on injecting tuberculin <https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/nvap/NVAP-Reference-Guide/Control-and-Eradication/Tuberculosis>)
 - i. Mid-cervical region
 - ii. Use separate disposable 1 ml plastic tuberculin syringes with 26 gauge 3/8 inch needles,
 - iii. Suggested locations PPD Avian: “above” or “ahead” ; PPD bovine: “below” or “behind”
 - iv. A new needle must be used for each injection.
 - v. Great care must be taken to not mix up the syringes
- h. If an error is made in injection (such as leakage, or partial injection), a new site can be prepared – Be sure to record the correct location in your notes
- 5. The same Field Veterinarian reads the CCT unless special permission is given by the tuberculosis epidemiologist to allow a different regulatory veterinarian to read the test
 - a. 72 hrs. +/- 6 hours from time of injection
 - b. Restrain the animal
 - c. Verify the animal identification
 - d. Measure and record the skin thickness of the sites post-injection using the approved calipers (measure to the nearest 0.5 mm for bovine, to the nearest 0.1 mm for cervids)
 - e. Calculate the change in skin thickness for each site (If using the MIM software with PDA, the calculation will be made for you.)
- 6. Fill out the VS 6-22C Tuberculin Test Report and VS6-22D Scattergram
 - a. It is imperative that the VS 6-22C form is properly completed. Failure to do so may result in lengthy follow-up to resolve errors including: inability to move animals, animal traces to find misidentified animals, and in some cases invalid TB tests.
 - b. In order to match the CCT with the correct initial test report, a copy of the practitioner’s completed VS 6-22 should be included with the CCT paperwork that is submitted to DATCP. Review the practitioner’s VS 6-22 for completeness and accuracy prior to filling out the VS 6-22C so errors can be addressed as soon as possible. Errors in animal ID are most common.
 - c. Check for common errors on the VS 6-22C and VS 6-22D
 - i. Address – owner’s name and mailing address. If animal location differs, please note in the remarks section or divide the owner address section to include both addresses and explain where the animals reside.

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- ii. Verify and include CFT or SCT injection date.
 - iii. Ensure CCT date is accurate.
 - iv. Retest – should almost always be “yes”. Discrepancies show up most frequently on MIMS forms
 - v. Double check tuberculin serial numbers
 - vi. Verify official ID - make sure official ID on animals matches the ID provided by the practitioner on the VS 6-22. Animal ID must be provided in writing (not via telephone) and is best done by requiring the veterinarian to send a scan to you prior to the CCT or to leave the completed copy of the 6-22 for you to reference when conducting CCT testing
 - vii. Age – verify age on VS 6-22 provided by the veterinarian matches the VS 6-22C. If ages don't match, please attach age statement in remarks.
 - viii. Double check calculations for PPD changes. Errors carry over to form 6-22D.
 - ix. Previous CFT or SCT response- “S” for each animal on the VS 6-22C. Previous test date is also entered on the VS 6-22D.
7. Determine if the quarantine can be released.
- a. If CCT is negative for all animals, release the quarantine (see DIS-021 Completing, Issuing, and Serving an Order of Quarantine) and give the original quarantine release to the owner – It is helpful to make a note somewhere on the release about the reason for release, such as “CCT negative on (date)”
 - b. If CCT is not negative for all animals, keep the herd quarantine in place.
 - i. Contact the tuberculosis epidemiologist or designee for further instructions.
 - ii. More in-depth epidemiological investigation and testing will be required – see CCT suspect and reactor procedures.
 - iii. The owner will need to implement contingency plans to account for the inability to move animals off of the premises during the quarantine (calves, cull cows, cattle going to next stage of production)
8. Distribute the VS 6-22C to the proper parties
- a. Originals are sent by mail, or scanned originals are emailed to DAH office, within 10 calendar days of completion of the test:
 - i. VS 6-22C test record (Part 1 – Federal Office copy)
 - ii. VS6-22D scattergram
 - iii. Copy of the quarantine release
 - iv. Copy of the practitioner's VS 6-22 that corresponds to the VS 6-22C
 - b. Owner - copy of VS 6-22C and quarantine release
 - c. Private practice veterinarian - copy of VS 6-22C
 - d. Additional copies of the VS 6-22 C labeled “Part 2- State Office, and Part 4- Regional Epidemiologist” are no longer sent to the office. These copies may be retained by the Field Veterinarian, or distributed to the private practitioner, or destroyed.
9. Upon receipt of the VS6-22 CCT forms in the office, a case assignment will be initiated and closed for the test case.