

Mycobacterium bovis – *Mycobacterium tuberculosis* Antibody Test Kit DPP[®] VetTB Assay for Cervids

A Rapid Immunochromatographic Kit for the Detection of Antibodies to *Mycobacterium bovis*
in Serum of Elk / Red Deer, Fallow Deer, and White-tailed Deer

FOR *IN VITRO* VETERINARY DIAGNOSTIC USE
READ INSTRUCTIONS FOR USE CAREFULLY BEFORE PERFORMING TEST

INTENDED USE

The DPP VetTB Assay is a single-use immuno-chromatographic test for the detection of antibodies to *Mycobacterium bovis* in serum of Elk / Red Deer (*Cervus elaphus*), Fallow Deer (*Dama dama*), and White-tailed Deer (*Odocoileus virginianus*). The test is used for the diagnosis of tuberculosis (TB) in conjunction with other diagnostic methods.

If specific antibodies are present in the sample, the expected test result is reactive. A reactive result is suggestive of active TB. In the absence of antibodies, the expected test result is nonreactive.

SUMMARY AND EXPLANATION

TB is considered to be one of the most important bacterial diseases due to its ability to spread quickly and to affect multiple host species. Infection of various cervid species with *M. bovis*, the etiologic agent of bovine TB, poses a serious threat to the livestock and wildlife. Timely identification of infected animals and/or herds is key in surveillance and control of bovine TB [1].

The current method for ante-mortem TB screening in cervids is the intradermal tuberculin test. This test has several limitations including suboptimal diagnostic accuracy and reproducibility, the need for repeated recaptures to administer and read results, and frequent animal injuries due to restraining procedures [2-3].

Serological methods constitute an attractive alternative, as they are relatively simple, inexpensive, non-invasive and they do not depend on detection of mycobacteria or their products. None of the existing TB tests alone is sufficient to definitively diagnose disease in live cervids. Therefore, new TB diagnostic algorithms are being developed, in which serological assays may play an important role.

Rapid detection of *M. bovis*-infected Elk / Red Deer, Fallow Deer, and White-tailed Deer by the Chembio DPP VetTB Assay serves as an effective diagnostic tool because it has high accuracy, is user-friendly, safe, and easy to perform [2-4] (see PERFORMANCE CHARACTERISTICS below).

PRINCIPLE OF TEST

The Chembio DPP VetTB Assay is based on immuno-chromatographic technology. The test employs two recombinant antigens, an *M. tuberculosis* and an *M. bovis* antigen, which are separately immobilized on the membrane solid phase. It also utilizes recombinant Protein A/G conjugated to colloidal gold particles for antibody detection. The DPP VetTB Assay for Cervids uses serum. The sample is applied to the SAMPLE+BUFFER well with the buffer. After the sample and buffer have migrated onto the test strip additional buffer is added to the BUFFER well. The buffer facilitates the lateral flow of the released products and promotes the binding of antibodies to the antigens. If present, the antibodies bind to the gold conjugated antibody binding protein. In a reactive sample, the dye conjugated-immune complex migrates on the nitrocellulose membrane and is captured by the antigens immobilized in the TEST (1 2) area producing a pink/purple line. In the absence of detectable antibody, no specific immune complex would be formed on the test line, and, therefore, no pink/purple line would appear in the TEST (1 2) area. Unbound conjugated gold particles continue to migrate along the membrane and produce a pink/purple line in the CONTROL (C) area. This procedural control serves to demonstrate that the reagents have been properly applied and have migrated through the device.

MATERIALS PROVIDED

Each kit contains the following items:

- 20 DPP VetTB test devices
- 1 DPP VetTB buffer vial (6mL)
- 1 Product insert

Additional Material Required But Not Provided

- Clock, watch or other timing device
- Disposable gloves
- Biohazard disposal container
- Collection devices for specimens
- Pipettor capable of delivering 5µL of sample

STORAGE AND STABILITY

The DPP VetTB Assay should be stored at 2 to 30°C in the original sealed pouch. The diluent should be stored in the original vial at 2 to 30°C. The kit is stable until the date printed on the box label and/or pouch.

NOTE: Do not use expired test kits.

CAUTION: Do not freeze test kits.

PRECAUTIONS

1. The test is designed FOR *IN VITRO* DIAGNOSTIC USE only. Use the test only in accordance with instructions supplied with the kit.
2. Handle all specimens as recommended for any potentially infectious serum or blood specimen in the CDC-NIH manual, *Biosafety in Microbiological and Biomedical Laboratories*, 4th ed., 1999.
3. Use suitable protective clothing (gloves, lab coat, safety glasses) when handling samples or test devices after samples have been applied. Avoid any contact between hands, eyes, nose or mouth during specimen collection and testing.
4. Do not pipette any material by mouth. Do not smoke, eat or drink in areas where specimens or kit material are kept.
5. All testing should be performed at a temperature of 18 to 30°C.
6. After the completion of the assay, carefully dispose of materials treating them as biohazardous waste.
7. Do not use expired test kits. Do not freeze test kits.
8. Do not mix reagents from different kit lots.

SPECIMEN COLLECTION

The DPP VetTB Assay for Cervids can be performed on serum only.

Serum: Serum is obtained from whole blood collected aseptically by venipuncture into a clean tube without anticoagulant. Allow the blood to clot at room temperature, centrifuge at 2000 rpm for 10 minutes at room temperature, 18 to 30°C, and separate the serum from the clot within 24 hours of blood collection.

Samples perform best when tested immediately after collection. Specimens should be immediately refrigerated at 2 to 8°C following collection and can be used up to 3 days. If testing within 3 days is not possible, the serum specimens should be frozen at -20°C or colder until use. Avoid repeated freezing and thawing.

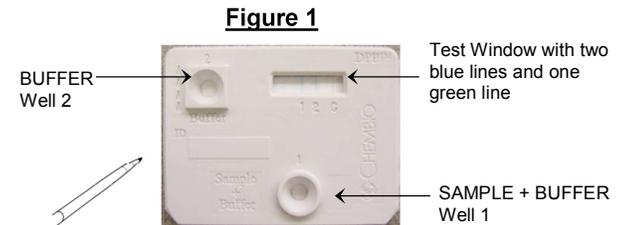
NOTE: If specimens are to be shipped, they should be packed in compliance with regulations covering the transportation of etiologic agents. Serum specimens should be shipped refrigerated with cold packs or wet ice. Serum samples may be shipped frozen in dry ice.

TEST PROCEDURE

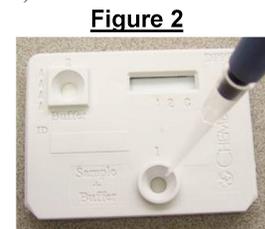
1. If test samples are refrigerated or frozen, remove them from the refrigerator or freezer and allow them to come to a temperature of 18 to 30°C before testing.
2. Remove the required number of DPP VetTB Assay devices from their pouches and place the devices on a flat surface area. It is not necessary to remove the desiccant from the package.

NOTE: If desiccant packet is missing, DO NOT USE, discard the test device and a new test device should be used.

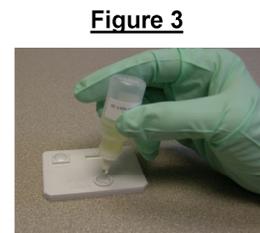
3. Label test units with sample names and/or identification numbers. (see Figure 1 below)



4. Using a 5 µl pipette, release the specimen carefully to the center of the round SAMPLE+BUFFER Well 1. (See Figure 2 below)

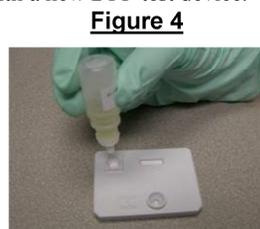


5. Once the specimen has been applied to the SAMPLE+BUFFER Well 1, remove the cap, invert the buffer bottle, hold it vertically over the SAMPLE+BUFFER Well 1, and add 2 drops (~65 µl) of the buffer slowly into SAMPLE+BUFFER well. (See Figure 3)



6. **Wait 5 minutes**, and then add 4 drops of the buffer to the square BUFFER Well 2. (See Figure 4 below.)

NOTE: The blue and green colored lines should have disappeared from the rectangular TEST and CONTROL window. If not, discard the test device and repeat the procedure with a new DPP test device.



7. Read the test result 15 minutes after the addition of the buffer into the BUFFER Well 2. In some cases a test line may appear in less than 15 minutes; however, 15 minutes are needed to report a non-reactive result. **Do not read results after 25 minutes from addition of Sample+Buffer to Well 1.**

- After reading and recording test results, discard the used test devices and any other test materials into a biohazard waste container.

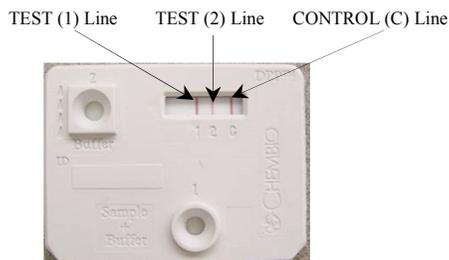
QUALITY CONTROL

A pink/purple colored line should always appear in CONTROL (C) area if the test has been performed correctly and the device is working properly. It serves as an internal test procedural control.

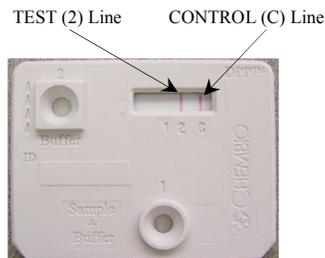
INTERPRETATION OF RESULTS

Reactive Result

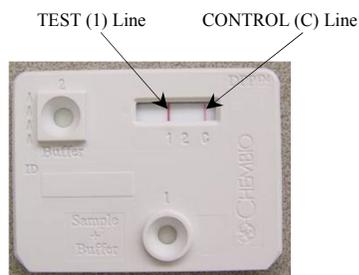
- Three pink/purple lines, one line in the TEST (1) area, one line in the TEST (2) area, and one line in the CONTROL area, are visible. This indicates that *Mycobacterium bovis* antibodies were detected in the sample.



- A pink/purple TEST (2) line and a pink/purple CONTROL line are visible. This indicates that *Mycobacterium bovis* antibodies were detected in the sample.



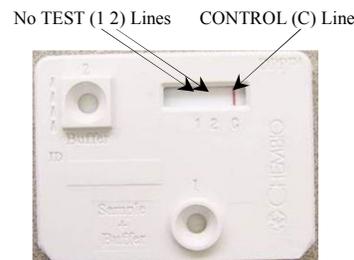
- A pink/purple TEST (1) line and a pink/purple CONTROL line are visible. This indicates that *Mycobacterium bovis* antibodies were detected in the sample.



NOTE: Intensities of the TEST and CONTROL lines may vary. Test lines are considered reactive regardless of intensity.

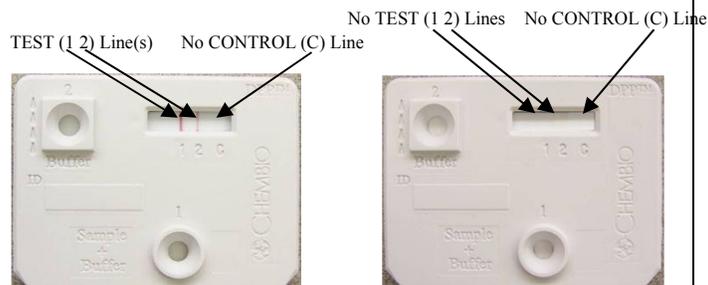
Nonreactive Result

Only a pink/purple CONTROL (C) line is visible. The sample contains no detectable antibodies to *Mycobacterium bovis*. A nonreactive result does not preclude the possibility of TB infection.



Invalid Result

A pink/purple line should always appear in the CONTROL (C) area, whether or not a line appears in the TEST area. If there is no distinct pink/purple line in the CONTROL (C) area, the test is invalid and should be repeated using a new device.



LIMITATIONS OF THE PROCEDURE

- The Chembio DPP VetTB Assay is designed for detecting antibodies against *M. bovis* only from cervid Elk / Red Deer, Fallow Deer, and White-tailed Deer serum samples. Any other body fluids or pooled specimens should not be used.
- Test results must be read between 15-20 minutes after the addition of the buffer to the square BUFFER Well 2.
- Do not open the sealed foil pouch until just prior to use.
- Do not use kit contents beyond labeled expiration date.
- Read results in a well-lit area.
- A reactive result suggests the presence of antibodies to *M. bovis*. The definitive diagnosis will require confirmation by other methods, such as culture and/or histopathology.
- For a reactive result, the intensity of the test line does not necessarily correlate with the titer of antibody in the specimen.
- A non-reactive result does not preclude the possibility of exposure to TB or infection with TB. An antibody response to recent exposure may take several months to reach detectable levels.

PERFORMANCE CHARACTERISTICS

Highly specific antibody-binding proteins of *M. bovis* are used in the DPP VetTB Assay to provide rapid and accurate detection of TB in Elk / Red Deer, Fallow Deer, and White-tailed Deer.

Sensitivity and Specificity

The sensitivity of the DPP VetTB Assay was determined by testing 265 serum samples from culture positive Elk / Red Deer, Fallow Deer, and White-tailed Deer. Of these samples, 197 were reactive with the DPP VetTB Assay (Table 1).

The specificity of the DPP VetTB Assay was determined by testing serum samples from 1243 culture negative Elk / Red Deer, Fallow Deer, and White-tailed Deer. Of these samples, 1212 were non-reactive with the DPP VetTB Assay (Table 2).

Table 1. Diagnostic Sensitivity of DPP VetTB Assay

Species	DPP VetTB Reactive	Culture Positive
Elk / Red Deer	78	96
Fallow Deer	78	106
White-tailed Deer	41	63
Total	197	265

Table 2. Diagnostic Specificity of DPP VetTB Assay

Species	DPP VetTB Nonreactive	Culture Negative
Elk / Red Deer	312	318
Fallow Deer	283	294
White-tailed Deer	617	631
Total	1212	1243

REPRODUCIBILITY STUDIES

Reproducibility was evaluated at three independent laboratories using two serials of DPP VetTB Assay. A reference panel of 40 blindly-coded samples representing negative, weakly reactive and strongly reactive sera were tested 3 times on 3 different days. The compiled results from 3 laboratories demonstrated 98.6% accuracy.

REFERENCES

1. Palmer, M.V. 2007. Tuberculosis: a reemerging disease at the interface of domestic animals and wildlife. *Curr. Top. Microbiol. Immunol.* 315: 195-215.
2. Waters, W. R., G. E. Stevens, M. A. Schoenbaum, K. A. Orloski, S. Robbe-Austerman, N. B. Harris, S. M. Hall, B. V. Thomsen, A. J. Wilson, R. E. Brannian, J. T. Nelson, S. Schafer, J. Esfandiari, M. Dutton, R. Greenwald, and K. P. Lyashchenko. 2011. Bovine tuberculosis in a nebraska herd of farmed elk and fallow deer: a failure of the tuberculin skin test and opportunities for serodiagnosis. *Vet. Med. Int.* 2011: 953985.
3. Nelson, J.T., K.A. Orloski, A.L. Lloyd, M. Camacho, M.A. Schoenbaum, S. Robbe-Austerman, B.V. Thomsen, and S.M. Hall. 2012. Evaluation of serodiagnostic assays for *Mycobacterium bovis* infection in elk, White-tailed deer, and reindeer in the United States. *Vet. Med. Int.* 2012: 563293.

4. Buddle, B. M., T. Wilson, M. Denis, R. Greenwald, J. Esfandiari, K. P. Lyashchenko, S. Liggett, and C. G. Mackintosh. 2010. Sensitivity, specificity, and confounding factors of novel serological tests used for the rapid diagnosis of bovine tuberculosis in farmed red deer (*Cervus elaphus*). *Clin Vaccine Immunol.* 17: 626-630.

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ORDERING INFORMATION

Cat #
65-9150-0

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DPP® VetTB 20 Test Kit



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