

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

A Rapid Immunochromatographic Test for the Detection of Antibodies to *Mycobacterium tuberculosis* and *Mycobacterium bovis* in Elephant Serum, Plasma or Whole Blood

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

INTENDED USE

The DPP VetTB Assay is a single use immunochromatographic rapid test for the detection of antibodies to *Mycobacterium tuberculosis* and *Mycobacterium bovis* in serum, plasma or whole blood from African elephants (*Loxodonta africana*) and Asian elephants (*Elephas maximus*). The test is used as an aid in the diagnosis of active 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

Tuberculosis (TB) in elephants is a re-emerging zoonotic disease caused primarily by *Mycobacterium tuberculosis* and, in some cases, by *Mycobacterium bovis* [1]. The only USDA-recommended definitive diagnostic test to detect TB in live elephants is mycobacterial culture of trunk wash samples. However, this method has poor diagnostic sensitivity, as it can only identify animals with extensive shedding of the organism that typically occurs late in the course of disease [2]. Rapid detection of infected elephants is a crucial prerequisite for more effective control of TB, as early diagnosis allows timely isolation and/or initiation of chemotherapy [1-3].

Serological methods constitute an attractive alternative as they are relatively simple, inexpensive, non-invasive, and they do not depend on detection of mycobacteria [3-4]. None of the existing TB tests alone is sufficient to diagnose disease. Therefore, new TB diagnostic algorithms are being developed, in which serological assays may play an important role [3-5] (see PERFORMANCE CHARACTERISTICS below).

The use of Chembio DPP VetTB Assay is an effective approach for rapid animal-side identification of elephants infected with *M. tuberculosis* or *M. bovis*, as this test is highly accurate, user-friendly, safe, and easy to perform.

PRINCIPLE OF TEST

The Chembio DPP VetTB Assay is based on immunochromatographic 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 uses serum, plasma or whole blood. 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 can be performed on whole blood, serum or plasma.

Whole Blood: Collect whole blood into tubes containing heparin or EDTA. Be sure to thoroughly mix whole blood by inverting capped tube several times just prior to testing. Follow test procedure instructions.

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.

Plasma: Collect whole blood with anticoagulant (heparin, EDTA or sodium citrate), centrifuge at 2000 rpm for 10 minutes at room temperature, 18 to 30°C, and isolate the plasma supernatant.

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 specimens should be frozen at -20°C or colder until use. Avoid repeated freezing and thawing. **DO NOT FREEZE WHOLE BLOOD.**

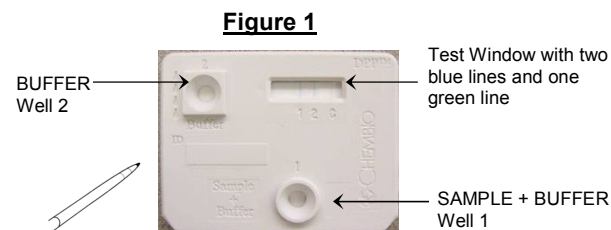
NOTE: If specimens are to be shipped, they should be packed in compliance with regulations covering the transportation of etiologic agents. Venous whole blood, serum and plasma specimens should be shipped refrigerated with cold packs or wet ice.

TEST PROCEDURE

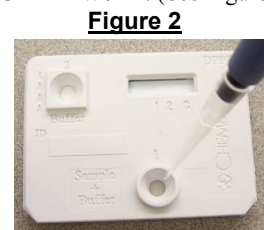
1. If test samples are refrigerated, remove them from the refrigerator and allow them to come to a temperature of 18 to 30°C before testing.
2. Remove the required number of DPP VetTB Assay devices from their pouches and place the devices on a flat surface area. It is not necessary to remove the desiccant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

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

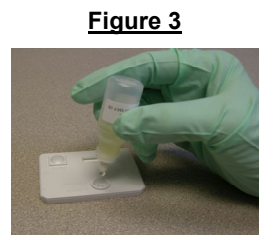
3. Label test units with sample names and/or identification numbers. (see Figure 1 below)



4. Using a 10µl disposable pipette (for whole blood) or a laboratory pipettor (for 5 µl of serum or plasma), release the specimen carefully to the center of the round SAMPLE+BUFFER Well 1. (See Figure 2 below)

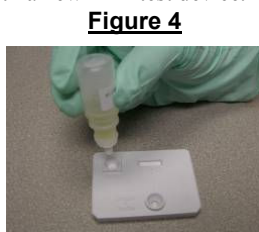


5. Once the specimen has been applied to the SAMPLE+BUFFER Well 1, remove the cap, invert the buffer bottle, hold it vertically over the SAMPLE+BUFFER Well 1, and add **2 drops (~65 µl)** of the buffer slowly into SAMPLE+BUFFER well. (See Figure 3)



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

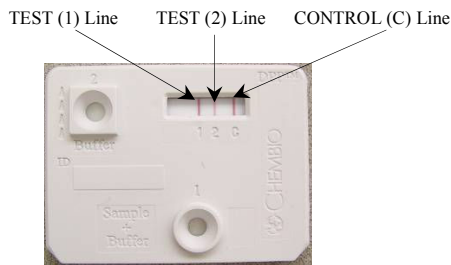
QUALITY CONTROL

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.

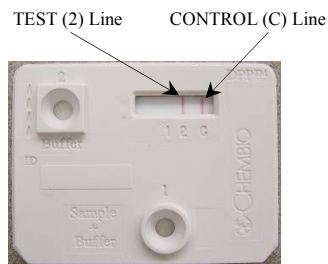
INTERPRETATION OF RESULTS

Reactive Result

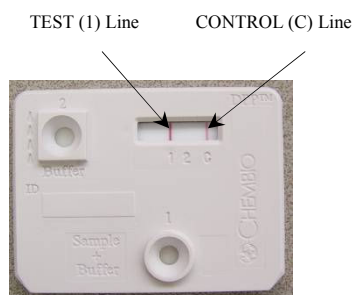
1. Three pink/purple lines, one line in the CONTROL area, one line in the TEST (1) area and one line in the TEST (2) area indicates a reactive result. This suggests that the sample is reactive for TB.



2. A pink/purple TEST (2) line and a pink/purple CONTROL line are visible. This suggests that the sample is reactive for TB.



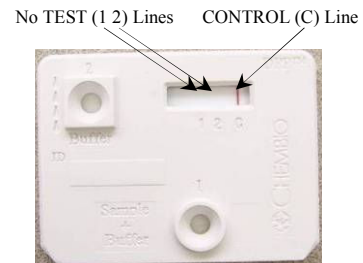
3. A pink/purple TEST (1) line and a pink/purple CONTROL line are visible. This suggests that the sample is reactive for TB or mycobacteriosis.



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

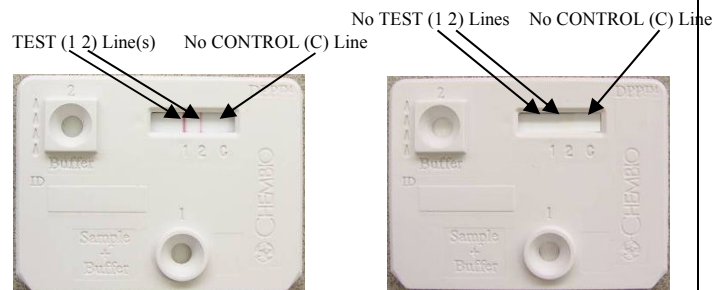
Nonreactive Result

Only a pink/purple CONTROL (C) line is visible. The sample contains no detectable antibody to both TB and mycobacteriosis antigens. 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

1. The Chembio DPP VetTB Assay is designed for detecting antibodies against *M. tuberculosis* and *M. bovis* only from elephant plasma, serum or whole blood. Any other body fluids or pooled samples or specimens from other than elephant species should not be used.
2. Test results must be read between 15-20 minutes after the addition of the buffer to the square BUFFER Well 2.
3. Do not use hemolyzed blood samples.
4. Blood specimens must be thoroughly mixed just prior to testing.
5. Do not open the sealed foil pouch until just prior to use.
6. Do not use kit contents beyond labeled expiration date.
7. Read results in a well-lit area.
8. A reactive result using the Chembio DPP VetTB Assay suggests the presence of antibodies to *M. tuberculosis* and/or *M. bovis*. The Chembio DPP VetTB Assay is intended as an aid in the diagnosis and treatment of TB in elephants.
9. For a reactive result, the intensity of the test line does not necessarily correlate with the titer of antibody in the specimen.
10. 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.
11. In treated elephants, interpret results with caution:
 - a. Treatment against TB may reduce antibody responses, thus resulting in non-reactive results in some cases.

- b. A reactive result may persist in infected and treated elephants for months and years even if an elephant is considered cured.

PERFORMANCE CHARACTERISTICS

Highly specific and sensitive antibody binding antigens are used in the DPP VetTB Assay. The diagnostic performance was compared to the standard USDA-recommended method, trunk wash culture, and the DPP VetTB Assay diagnostic performance was found to be superior [4-5].

Further, it was shown that both Asian and African elephants infected with *M. tuberculosis* or *M. bovis* could be detected by DPP VetTB Assay up to several years prior to finding positive culture in trunk washes [4-5].

Sensitivity and Specificity

Sensitivity of the DPP VetTB Assay was determined by testing 40 culture positive elephants. All 40 samples were reactive (Table 1).

The specificity of the DPP VetTB Assay was determined by testing serum, plasma, and/or whole blood samples collected from 147 trunk-wash culture negative elephants without history of TB. All 147 samples were non-reactive (Table 2).

Table 1.
Diagnostic sensitivity of DPP VetTB Assay

| Elephant species | Mycobacterial species isolated | DPP VetTB reactive |
|------------------|--------------------------------|--------------------|
| African | <i>M. tuberculosis</i> | 6/6 |
| African | <i>M. bovis</i> | 1/1 |
| Asian | <i>M. tuberculosis</i> | 33/33 |

Table 2.
Specificity studies of DPP VetTB Assay

| Elephant species | DPP VetTB Assay non-reactive | Trunk wash culture negative |
|------------------|------------------------------|-----------------------------|
| African | 79/79 | 79/79 |
| Asian | 68/68 | 68/68 |

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

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2. Lewerin, S.S., Olsson, S-L., Eld, K., Röken, B., Ghebremichael, S., Koivula, T., Källenius, G., and Bölske, G. (2005) Outbreak of *Mycobacterium*

tuberculosis infection among captive Asian elephants in a Swedish zoo. *Vet. Rec.* 156: 171-175. Lyashchenko, K.P., et al., (2006) Tuberculosis in elephants: antibody responses to defined antigens of *Mycobacterium tuberculosis*, potential for early diagnosis, and monitoring of treatment. *Clinical And Vaccine Immunology* 13: 722-732.

3. Greenwald, R., Lyashchenko, O., Esfandiari, J., Miller, M., Mikota, S., Olsen, J.H., Ball, R., Dumonceaux, G., Schmitt, D., Moller, T., Payeur, J.B., Harris, B., Sofranko, D., Waters, W.R., and Lyashchenko, K.P. (2009) Highly accurate antibody assays for early and rapid detection of tuberculosis in African and Asian elephants. *Clinical And Vaccine Immunology* 16:605-612.
4. Lyashchenko K.P., Greenwald R., Esfandiari J., Mikota S., Miller M., Moller T., Vogelnest L., Gairhe K.P., Robbe-Austerman S., Gai J., and Waters W.R. (2012) Field application of serodiagnostics to identify elephants with tuberculosis prior to case confirmation by culture. *Clin. Vaccine Immunol.* 19(8):1269-75.

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ORDERING INFORMATION

| Cat # | Product |
|-----------|------------------------|
| 65-9110-0 | DPP® VetTB 2 Test Kit |
| 65-9111-0 | DPP® VetTB 5 Test Kit |
| 65-9112-0 | DPP® VetTB 20 Test Kit |



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