**Mycobacterium bovis – Mycobacterium tuberculosis**

**Antibody Test Kit**

**ElephantTB STAT-PAK® Assay**

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 ElephantTB STAT-PAK Assay is a qualitative, single use, two-step, immunochromatographic screening 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*. The only USDA-recommended diagnostic test for TB in elephants is mycobacterial culture of trunk wash samples. However, there is a growing body of evidence indicating that this method has poor sensitivity, as it can only identify animals with extensive shedding of the organism that typically occurs late in the course of disease. Rapid detection of infected elephants is a crucial prerequisite for more effective control of TB, as early diagnosis allows timely initiation of chemotherapy [1-3].

Serological methods constitute an attractive alternative as they are simple, inexpensive, relatively non-invasive, and they do not depend on detection of mycobacteria [4-5]. 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 (see PERFORMANCE CHARACTERISTICS below).

The Chembio ElephantTB STAT-PAK Assay is a rapid immunochromatographic test for antibody detection that is safe, simple, and easy to perform.

**PRINCIPLE OF TEST**

The Chembio ElephantTB STAT-PAK Assay is based on immunochromatographic (lateral-flow) technology. The test employs a unique cocktail of recombinant *M. tuberculosis* proteins that are bound to the membrane solid phase. Blue latex particles conjugated with protein are used as the detection system. The ElephantTB STAT-PAK Assay can be used with serum, plasma or whole blood. Once a test sample is applied to the SAMPLE (S) well followed by the addition of a diluent, it flows laterally through the membrane strip. When it reaches the conjugate pad, antibodies, if present, bind to protein-latex conjugate and then the migrating immune complex binds to the antigens on the solid phase in the TEST (T) area producing a blue line. In the absence of antibodies there is no line in the TEST (T) area. The sample continues to migrate along the membrane and produces a blue line in the CONTROL (C) area demonstrating that the reagents are functioning properly.

**MATERIALS PROVIDED**

Each kit contains the following items:

- 50 ElephantTB STAT-PAK test devices
- 50 Disposable pipettes
- 2 Diluent vials (5mL each)
- 1 Product insert

**Additional Material Required But Not Provided**

- Clock, watch or other timing device
- Disposable gloves
- Biohazard disposal container
- Collection devices for specimens
STORAGE AND STABILITY
The ElephantTB STAT-PAK Assay should be stored at 8 to 30°C in the original sealed pouch. The diluent should be stored in the original vial at 8 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 ElephantTB STAT-PAK 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 used 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.
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 ElephantTB STAT-PAK 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 disposable pipette, draw the specimen to be tested (whole blood, serum or plasma), into the pipette being careful not to draw up any air and add one full drop (30 µl) of specimen onto the center of the SAMPLE (S) well. (See Figure 2 below)

5. Once the specimen has been applied to the SAMPLE (S) well, remove the cap, invert the diluent bottle and hold it vertically (not at an angle) over the SAMPLE well. Add the diluent slowly dropwise; add 3 drops (~100 µl) into SAMPLE (S) well. (See Figure 3)

6. Read results at 20 minutes after the addition of diluent. Do not read any results after 30 minutes. Refer to INTERPRETATION OF RESULTS section below.
7. Discard the used disposable pipette, test device and any other test materials into a biohazard waste container.
QUALITY CONTROL
A blue 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.

Good Laboratory Practice (GLP) recommends the use of control materials along with the test samples to ensure proper performance of the test kit. Positive and Negative serum or plasma based commercial controls should be used for this purpose. Use controls as per the TEST PROCEDURE instructions of this insert.

INTERPRETATION OF RESULTS

Nonreactive Result
One blue colored line in the CONTROL (C) area, with no visible colored line in the TEST (T) area indicates a nonreactive result. A nonreactive result at 20 minutes means that neither *Mycobacterium tuberculosis* nor *Mycobacterium bovis* antibodies were detected in the specimen. A nonreactive result does not preclude the possibility of TB infection.

Reactive Result
Two blue lines - one in the TEST (T) area and one in the CONTROL (C) area - indicate a reactive result. Intensities of the TEST and CONTROL lines may vary. Even a very faint line in the TEST (T) area of the device within 20 minutes is indicative of a reactive result. A reactive result means that *Mycobacterium tuberculosis* and/or *Mycobacterium bovis* antibodies were detected in the specimen.

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

COMPLETE TEST LINE
An incomplete line in the TEST (T) area should not be interpreted. The test should be repeated using a new device. If an incomplete line in the TEST (T) area occurs upon repeat test, further testing using other supplemental and/or confirmatory methods are indicated.

LIMITATIONS OF THE PROCEDURE
1. The 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. A reactive result suggests the presence of antibodies to *M. tuberculosis* and/or *M. bovis*.
3. For a reactive result, the intensity of the test line does not necessarily correlate with the titer of antibody in the specimen.
4. Reading nonreactive results earlier than 20 minutes or any results later than 30 minutes may yield erroneous results.
5. Do not use hemolyzed blood samples.
6. Blood specimens must be thoroughly mixed just prior to testing.
7. Be careful to add only 30 µL of specimen and 3 drops of diluent after applying the specimen to the SAMPLE (S) well.
8. Do not open the sealed test pouch until just prior to testing.
9. Do not use kit contents beyond labeled expiration date.
10. Read results in a well-lit area.
PERFORMANCE CHARACTERISTICS

Highly specific and sensitive antibody binding proteins are used in the ElephantTB STAT-PAK Assay. The diagnostic performance was compared to the standard USDA-recommended method, trunk wash culture, and the ElephantTB STAT-PAK Assay was found to be superior.

Further, it was shown that both Asian and African elephants infected with either M. tuberculosis or M. bovis could be detected by ElephantTB STAT-PAK Assay up to several years prior to finding positive culture in trunk washes [3].

Sensitivity and Specificity

Sensitivity of the ElephantTB STAT-PAK Assay was determined by testing 23 culture positive elephants. Of these samples, 23/23 were reactive by the Chembio ElephantTB STAT-PAK antibody test kit (Table 1). The specificity of the ElephantTB STAT-PAK Assay was determined by testing 131 serum, plasma, and/or whole blood samples. Of these samples 127/131 were reactive by the Chembio ElephantTB STAT-PAK antibody test kit (Table 2).

Table 1. Diagnostic sensitivity of ElephantTB STAT-PAK Assay

<table>
<thead>
<tr>
<th>Elephant</th>
<th>Mycobacterial species</th>
<th>ElephantTB STAT-PAK reactive</th>
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<tbody>
<tr>
<td>African</td>
<td>M. tuberculosis</td>
<td>3/3</td>
</tr>
<tr>
<td>African</td>
<td>M. bovis</td>
<td>1/1</td>
</tr>
<tr>
<td>Asian</td>
<td>M. tuberculosis</td>
<td>19/19</td>
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Table 2. Specificity studies of ElephantTB STAT-PAK Assay

<table>
<thead>
<tr>
<th>Elephant</th>
<th>ElephantTB STAT-PAK non-reactive</th>
<th>Trunk Lavage Culture negative</th>
</tr>
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<tbody>
<tr>
<td>African</td>
<td>58/58</td>
<td>58/58</td>
</tr>
<tr>
<td>Asian</td>
<td>50/54</td>
<td>54/54</td>
</tr>
<tr>
<td>Unknown</td>
<td>19/19</td>
<td>19/19</td>
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</tbody>
</table>

REPRODUCIBILITY STUDIES

Reproducibility was tested at three independent laboratories using three serials of ElephantTB STAT-PAK Assay. A reference panel of 30 blinded samples representing negative, weakly reactive and reactive were tested 3 different times on 3 different days. The compiled results from 3 laboratories demonstrated 98.5% accuracy.

REFERENCES


FOR MORE INFORMATION, CONTACT:

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ORDERING INFORMATION

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Product</th>
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<tr>
<td>60-9680-0</td>
<td>ElephantTB STAT-PAK® 5 Test Kit</td>
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<tr>
<td>60-9682-0</td>
<td>ElephantTB STAT-PAK® 20 Test Kit</td>
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<tr>
<td>60-9683-0</td>
<td>ElephantTB STAT-PAK® 50 Test Kit</td>
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