**Mycobacterium bovis – Mycobacterium tuberculosis**

**Antibody Test Kit, Code 5470.01**

**CervidTB STAT-PAK® Assay**

A Qualitative Screening Test Kit for the Detection of Antibodies to *Mycobacterium bovis* in Whole Blood and 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 CervidTB STAT-PAK Assay is a qualitative, single use, two-step, immunochromatographic screening test for the detection of antibodies to *Mycobacterium bovis* in whole blood and 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 indicative of 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 present due to its ability to spread quickly and to affect multiple host species. Infection of Elk / Red Deer, Fallow Deer or White-tailed Deer with *M. bovis*, the etiologic agent of bovine TB, poses a threat to wildlife and livestock. Timely identification of infected animals and / or herds is key in surveillance and control of bovine TB [1-3].

The current method for ante-mortem screening for TB in Elk / Red Deer, Fallow Deer, and White-tailed Deer 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.

Rapid detection of *M. bovis* infected Elk / Red Deer, Fallow Deer, and White-tailed Deer by the Chembio CervidTB STAT-PAK Assay serves as an effective diagnostic tool because it is user-friendly, safe, simple and easy to perform.

Serological methods constitute an attractive alternative, as they are simple, inexpensive, relatively non-invasive and they do not depend on detection of mycobacteria or their products. None of the existing Elk / Red Deer, Fallow Deer, and White-tailed Deer TB tests alone is sufficient to definitively diagnose disease. Therefore, new TB diagnostic algorithms are being developed, in which serological assays may play an important role (see PERFORMANCE CHARACTERISTICS below).

**PRINCIPLE OF TEST**

The Chembio CervidTB STAT-PAK Assay is based on immunochromatographic (lateral-flow) technology. The test employs a unique cocktail of recombinant *M. bovis* proteins that are bound to the membrane solid phase. Blue latex particles conjugated with protein are used as the detection system. The CervidTB STAT-PAK Assay can be used with whole blood and serum. Once a specimen 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 immobilized on the solid phase producing a blue line in the TEST (T) area. In the absence of antibodies there is no visible line in the TEST (T) area. The sample continues to migrate along the membrane and produces another blue line appearing in the CONTROL (C) area demonstrating that the reagents are functioning properly.

**MATERIALS PROVIDED**

Kit Contents:
- 50 CervidTB STAT-PAK test devices
- 50 Disposable pipettes
- 2 Diluent vial (5mL each)
- 1 Product insert

Additional Materials Required But Not Provided
- Clock, watch or other timing device
- Disposable gloves
- Biohazard disposal container
- Collection devices for specimens

**STORAGE**

The CervidTB 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 imprinted on the box label and / or pouch.

**CAUTION:** Do not freeze 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 human serum or blood specimen in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 5th ed., 2007.
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.
8. Do not mix reagents from different kit lots.

SPECIMEN COLLECTION

The CervidTB STAT-PAK Assay can be performed on whole blood or serum.

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.

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 (18 to 30°C), centrifuge at 2000 rpm for 10 minutes at 18 to 30°C, then separate the serum from the clot.

Samples perform best when tested immediately after collection. If tested the same day, whole blood may be kept at 18 to 30°C. Otherwise, whole blood specimens may be stored for up to 3 days at 2 to 8°C before testing. If testing within 3 days is not possible, serum specimens should be frozen at -20°C or colder until use. Avoid repeated freezing and thawing.

DO NOT FREEZE WHOLE BLOOD.
DO NOT USE HEMOLYZED SAMPLES.

NOTE: If specimens are to be shipped, they should be packed in compliance with regulations covering the transportation of etiologic agents. Whole blood and serum 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 CervidTB 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 devices with sample identification numbers (Figure 1).

4. Using a disposable pipette provided, draw the specimen into the pipette being careful not to draw up any air and add one drop (~20µL) of specimen onto the center of the SAMPLE (S) well (Figure 2). Alternatively, this step may be performed by using a laboratory pipettor for 20µL.

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

6. Read results at 20 minutes after the addition of diluent in a well lit area as described in INTERPRETATION OF RESULTS section below. Do not read any results after 30 minutes.

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.
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 no *M. bovis* antibodies were detected in the specimen. A nonreactive result does not preclude the possibility of *M. bovis* infection.

![Nonreactive Result](image)

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 bovis* antibodies were detected in the specimen.

![Reactive Result](image)

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.

![Invalid Result](image)

Incomplete 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.

![Incomplete Test Line](image)

LIMITATIONS

1. The assay is designed for detecting antibodies against *M. bovis* only from Elk /Red Deer, Fallow Deer, and White-tailed Deer whole blood and serum. Any other body fluids or pooled samples or specimens should not be used.
2. 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.
3. A nonreactive result does not rule out *M. bovis* infection (early stages, particularly) or recent exposure.
4. For a reactive result, the intensity of the test line does not necessarily correlate with the titer of antibody in the specimen.
5. Reading nonreactive results earlier than 20 minutes or any results later than 30 minutes may yield erroneous results.
6. Be careful to add only 20 µL of specimen and 3 drops of diluent after applying the specimen to the SAMPLE (S) well.
7. Do not open the sealed test pouch until just prior to use.
PERFORMANCE CHARACTERISTICS

Highly specific antibody binding proteins are used in the CervidTB STAT-PAK 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 CervidTB STAT-PAK Assay was determined by testing 410 whole blood and/or serum samples from culture positive Elk / Red Deer, Fallow Deer, and White-tailed Deer. Of these samples, 315 were reactive with the CervidTB STAT-PAK test kit (Table 1).

The specificity of the CervidTB STAT-PAK Assay was determined by testing whole blood samples from 3664 culture negative Elk / Red Deer, Fallow Deer, and White-tailed Deer. Of these samples, 3467 were non-reactive with the CervidTB STAT-PAK test kit (Table 2).

Table 1. Diagnostic Sensitivity of CervidTB STAT-PAK Assay

<table>
<thead>
<tr>
<th>Species</th>
<th>CervidTB STAT-PAK Reactive</th>
<th>Culture Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elk / Red Deer</td>
<td>113</td>
<td>133</td>
</tr>
<tr>
<td>Fallow Deer</td>
<td>115</td>
<td>140</td>
</tr>
<tr>
<td>White-tailed Deer</td>
<td>87</td>
<td>137</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>315</strong></td>
<td><strong>410</strong></td>
</tr>
</tbody>
</table>

Table 2. Diagnostic Specificity of CervidTB STAT-PAK Assay

<table>
<thead>
<tr>
<th>Species</th>
<th>CervidTB STAT-PAK Nonreactive</th>
<th>Culture Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elk/Red Deer</td>
<td>904</td>
<td>971</td>
</tr>
<tr>
<td>Fallow Deer</td>
<td>440</td>
<td>498</td>
</tr>
<tr>
<td>White-tailed Deer</td>
<td>2123</td>
<td>2195</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>3467</strong></td>
<td><strong>3664</strong></td>
</tr>
</tbody>
</table>

REPRODUCIBILITY STUDIES

Reproducibility was tested at three independent laboratories using three test serials. A reference panel of 30 blinded samples representing negative, weakly reactive and reactive specimens were tested by 3 different operators on 3 different days. The compiled results demonstrated 98.5% concordance.

REFERENCES