**LIO Detect® TB-ST Tuberculosis Rapid Test**

Rapid test for the qualitative detection of human IgG, IgA and IgM antibodies to *Mycobacterium tuberculosis* in serum, plasma or whole blood. Intended for professional in vitro diagnostic use.

**INTENDED USE**

The LIO Detect® TB-ST Tuberculosis Rapid Test is an in vitro diagnostic rapid test for the qualitative detection of IgG, IgA and IgM antibodies to *Mycobacterium tuberculosis* in serum, plasma or whole blood within 20 minutes.

This test is intended for the rapid diagnosis of active tuberculosis (TB) and is not suitable for contact screening of populations.

**INTRODUCTION / FIELD OF APPLICATION**

Human TB has become a global disease with its re-emergence in the Western countries in the last decades. According to WHO, more than 30% of the world’s population is estimated to be infected with the TB bacterium, *M. tuberculosis*. TB is predominantly a disease of the respiratory tract, but can also affect other organs. People who are suffering from active pulmonary tuberculosis are highly infectious. The spread of TB takes place by coughing and sneezing. TB kills yearly about 2 million people.

Although, the TB bacterium was identified more than 100 years ago, the diagnostic methods, which are currently available, suffer from high price, poor sensitivity and a specificity and are mostly time consuming. The diagnosis of TB is usually made based on a combination of several laboratory tests. The LIO Detect® TB-ST Tuberculosis Rapid Test is suitable for the qualitative detection of human IgG, IgA and IgM antibodies within 20 minutes. This test is an additional tool for rapid diagnosis of active TB using small amount of sample and the results are available on site.

**PRINCIPLE OF THE TEST**

The LIO Detect® TB-ST Tuberculosis Rapid Test is a membrane based test for the qualitative detection of IgG, IgA and IgM antibodies to *M. tuberculosis* in serum, plasma or whole blood.

The test consists of one test strip, which is integrated in a test cassette. The test strip consists of a control line (C) and a test zone (T). The remaining complex migrates further across the membrane to the control zone (C’). Upon the appearance of the test line, the test is considered positive.

**SUPPLIED MATERIALS**

**Packaging sizes:**

REF: 3010 (10 Tests)

10 test cassettes and 1 dropper bottle containing 3.5 mL of LIO Detect® TB-ST Diluent.

**TEST COMPONENTS**

- LIO Detect® TB-ST Diluent: dropper bottle containing dilution buffer - 3.5 mL
- Test cassette: individually sealed in an aluminum bag with a single use pipet
- 1 Instructions for use

**MATERIALS NEEDED BUT NOT SUPPLIED**

- Stop watch.
- Containers for sample collection. We recommend using standard containers for blood collection.

**PREPARATION OF REAGENTS**

All reagents are ready-to-use. No further preparation of reagents is necessary.

**STABILITY AND STORAGE CONDITIONS**

Store the test at 2 - 25°C. Unopened kit components (aluminum bags and LIO Detect® TB-ST Diluent) are stable until the expiry date. The expiry date is printed on the labels of the aluminum bag, the LIO Detect® TB-ST Diluent and the outer packaging. Do not use if the diluent bag is damaged. DO NOT FREEZE or expose to temperatures above 30°C.

**Aluminum pouch with test cassette:** Keep the test in an unopened aluminum bag at 2 - 25°C.

**Opened aluminum bag:** Use test cassette within 8 hours!

**LIO Detect® TB-ST Diluent (dilution buffer):** Store the LIO Detect® TB-ST Diluent at 2 - 25°C. Unopened LIO Detect® TB-ST Diluent is stable until the expiry date. After first opening the LIO Detect® TB-ST Diluent is stable until the expiry date, if the bottle is tightly closed after every usage.

**WARNINGS AND PRECAUTIONS**

- In accordance with Good Laboratory Practice (GLP), all laboratory devices employed should be regularly checked for the accuracy and precision.
- Use all reagents within the expiry period (printed on the label).
- Do not use reagents from different kit lots or batch.
- Avoid contamination of the reagents. Do not use the same container for several samples! Use separate single-use pipettes for each sample (included in the kit).
- Lipemic, haemolytic or bacterially contaminated samples should not be used.
- Avoid the use of turbid samples which may be contaminated with bacteria.
- Avoid repeated freezing and thawing of the samples. It could lead to denaturation of the antibodies.
- For in vitro diagnostic use only! Do not ingest or swallow! Do not eat, drink and smoke in the laboratory! Do not work without wearing protective clothing (gloves, safety glasses and lab coat)! Avoid contact of test reagents with skin, eye or mucosa.
- All kit components should be considered as infectious agents. Decominate and dispose of residues of kit reagents and samples in accordance to local regulations, e.g. by autoclaving or using a disinfecting solution.
- Avoid contact of test reagents and samples to skin, eye and mucosa. Avoid touching of the membrane in the result window of the test device with your fingers (danger of contamination).
- Do not pipette samples and LIO Detect® TB-ST Diluent directly onto the membrane in the result window of the test device.
- For single-use only. The test is sensitive to moisture. Do not use if the outer packaging (aluminum bag) is damaged. After opening the aluminum bag, it must be used within 8 hours.

**SAMPLE COLLECTION AND PREPARATION**

The LIO Detect® TB-ST Tuberculosis Rapid Test is suitable for the detection of IgG, IgA and IgM antibodies in serum, plasma or whole blood. The test works best with fresh samples.

**Collection of whole blood from the vein:**

Take the sample under standard laboratory conditions (aseptically, avoid haemolysis).

**Collection of whole blood from the fingertip:**

- Disinfect your hands.
- Ask the patient to sit or to stretch himself out.
- Use disposable gloves.
- Disinfect a puncture site with a skin disinfectant.
- Wait for exposure and drying time of the disinfectant.
- Puncture the skin with a sterile lancet.
- Massage the hand towards the fingertip (Caution! Do not touch the puncture site! Avoid strong pressure!)
- Discard the first drop and gently massage the hand from wrist to fingers to cause the formation of a drop of blood.
- Keep the puncture site downward (horizontal or slightly inclined) and take the drop of blood with a single use capillary or pipet. Try to touch only the sealed blood and avoid air bubbles.

**Serum, plasma or whole blood:** Separate as soon as possible from the red blood cells (e.g. by centrifugation).

If the test cannot be performed immediately after the sampling, the samples can be stored for up to 2 days (48 hours) at 2 - 8°C, longer storage, the whole blood must be centrifuged (separate serum or plasma from red blood cells). Serum and plasma can be stored at temperatures below -20°C. Frozen samples must be thawed prior to testing and well mixed. Avoid repeated freezing and thawing of samples!

**TEST PROCEDURE**

**Test procedure time** is 20 minutes

**Serum or plasma:**

1. Take the required number of test cassettes from the packaging kit. Remove the aluminum bag and place the cassette / s on a clean, non-absorbent flat surface.
2. Pipette two drops of the sample into the sample well (S) on the cassette. Use the single-use pipet contained in the aluminum bag or alternatively a microliter pipet (sample volume 80 µL ± 10 µL).
3. Add 2 drops of LIO Detect® TB-ST Diluent into the sample well (S).
4. Start stop watch after addition of LIO Detect® TB-ST Diluent and read the results after 20 minutes.

**Whole blood:**

1. Take the required number of test cassettes from the packaging kit. Remove the aluminum bag and place the cassette / s on a clean, non-absorbent flat surface.
2. Pipette one drop of the sample into the sample well (S) on the cassette. Use the single-use pipet contained in the aluminum bag or alternatively a microliter pipet (sample volume 40 µL ± 10 µL).
3. Add 3 drops of LIO Detect® TB-ST Diluent into the sample well (S).
4. Start stop watch after addition of LIO Detect® TB-ST Diluent and read the results after 20 minutes.
QUALITY CONTROL

The LIODetect®TB-ST Tuberculosis Rapid Test contains an internal control. A colored line in the control zone ("C") is considered as an internal procedural control. It confirms sufficient sample volume and correct test procedure. A clear background is an internal negative procedural control. If a background color appears in the result window and thereby the readability of the test results will be affected, the result may be invalid.

PERFORMANCE CHARACTERISTICS

To determine the diagnostic sensitivity and specificity, 476 samples were evaluated with the LIODetect®TB-ST Tuberculosis Rapid Test by internal study (in-house study). Additionally 192 samples were measured by an independent external study in Russia.

The results of the LIODetect®TB-ST Tuberculosis Rapid Test were compared with clinical outcomes. As gold standard for the diagnosis of TB, a pathogen detection based on cultivation, microscopy, X-ray and/or PCR was defined (positive control group). The panel of negative control group implies samples of clinically healthy donors and patients with other diseases. The results of the in-house study are summarized in Table 1. The results of external study are shown in Table 2.

Tab. 1: Results of in-house study. Negative control group (no TB): samples from healthy donors and patients with other diseases, IGRA positive or unknown, different countries / positive control group (TB): microscopy positive or negative culture confirmed cases from different countries.

<table>
<thead>
<tr>
<th>Method</th>
<th>Clinical diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIODetect® TB-ST</td>
<td>negative</td>
</tr>
<tr>
<td>positive</td>
<td>53</td>
</tr>
<tr>
<td>Total results</td>
<td>153</td>
</tr>
<tr>
<td>% Concordance</td>
<td>65.36</td>
</tr>
</tbody>
</table>

Tab. 2: Results of external study (Russia). Negative control group (no TB): negative for X-ray for TB; Mantoux test, Bacteriology and HIV / positive control group (TB): microscopy positive or negative, X-ray confirmed cases.

<table>
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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>LIODetect® TB-ST</td>
<td>negative</td>
</tr>
<tr>
<td>positive</td>
<td>25</td>
</tr>
<tr>
<td>Total results</td>
<td>92</td>
</tr>
<tr>
<td>% Concordance</td>
<td>72.83</td>
</tr>
</tbody>
</table>

LIMITATIONS

Follow the instructions of the test procedure and interpretation of results carefully!

The LIODetect®TB-ST Tuberculosis Rapid Test has been developed to detect IgG, IgA and IgM antibodies to M. tuberculosis in serum, plasma or whole blood. It is intended for professional in vitro diagnostic use only. For the measurement of other body fluids, this test has not been validated and results may be incorrect.

The test is specific for active TB. The test is not suitable for the detection of so-called latent TB (LTB) or for contact screening of populations. Cross reactions may occur rarely if infections with other pathogenic mycobacteria are present. If only test line 2 (T2) appears and no further indications for active TB are present, an infection with other Mycobacteria should be excluded. The reaction of line 2 may also indicate current or past TB. Hence, we suggest the positive reaction of line 2 as an indication of mycobacterial infection or disease.

A definitive clinical diagnosis should be based on an evaluation of all clinical and laboratory findings by a doctor, and not only by the results of one single test. If a patient sample was tested as positive, more confirmatory tests should be performed (e.g. microscopy, culture results, serology, PCR, clinical symptoms, IGRA-cytokine detection). For a final diagnosis, include all information available for a patient.

Likewise, a negative test result does not exclude a possible TB infection or disease.

Note that doubtful results need further confirmation by performing tests like an interferon gamma release assay (IGRA), culture and PCR analysis to confirm or exclude a possible infection.

Recent or ongoing treatment for TB may lead to faulty results. Antibody levels in the blood may diminish rapidly after treatments with anti-TB antibiotics. Sometimes antibody levels can be so low in patient’s blood samples, that antibodies cannot even be detected at all, in blood, plasma or serum - even if an infection or disease is present.

Interfering substances:

Samples are spiked by the following interfering substances:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Testing concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>20 mg/dL</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>20 mg/dL</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>20 mg/dL</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>60 mg/dL</td>
</tr>
<tr>
<td>Caffeine</td>
<td>20 mg/dL</td>
</tr>
<tr>
<td>EDTA</td>
<td>20 mg/dL</td>
</tr>
<tr>
<td>Glucose</td>
<td>2000 mg/dL</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>500 mg/dL</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>200 mg/dL</td>
</tr>
<tr>
<td>Sodium heparin</td>
<td>3 mg/dL</td>
</tr>
<tr>
<td>Lithium heparin</td>
<td>3 mg/dL</td>
</tr>
</tbody>
</table>

No interference is observed for one of the substances tested.

LITERATURE


For in-vitro diagnostic use only