LIODetect®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.

REF: 3010_EN **Rev. 3.0 / 181019**

INTENDED USE

The **LIODetect®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 specificity and are mostly time consuming. The diagnosis of TB is usually made based on a combination of several laboratory tests. The **LIODetect®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 **LIODetect®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. This test strip consists of a special antibody-binding protein, coupled to colored particles (conjugate), and a membrane with two test lines and one control line. The test lines contains TB antigens, the control line consists of an antibody-binding protein.

After the sample (serum, plasma or whole blood) is pipetted into the sample well (S) followed by the LIODetect®TB-ST Diluent, the diluted sample passes through the conjugate and the antibodies in the sample bind to the conjugate. The antibody-conjugate complex migrates due to the capillary action to the site of the membrane where the TB antigens are immobilized (test lines). If antibodies against tuberculosis are present in the sample, these bind to the test lines.

Then one or two colored lines appear in the **test zone ("T")**. The remaining complex migrates further across the membrane to the **control zone ("C")**. Again a colored line appears, indicating that the test was performed correctly.

SUPPLIED MATERIALS

Packaging sizes:

REF: 3010 (10 Tests): 10 test cassettes and 1 dropper bottle containing 3.5 mL of LIODetect®TB-ST Diluent.

TEST COMPONENTS

- LIODetect®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

Note: Pictures may differ from the original.

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 (aluminium bags and LIODetect®TB-ST Diluent) are stable until the expiry date. The expiry date is printed on the labels of the aluminium bag, the LIODetect®TB-ST Diluent and the outer packaging. Do not use if the aluminum bag is damaged. **DO NOT FREEZE** or expose to temperatures above 30°C.

Aluminium pouch with test cassette: Keep the test in unopened aluminium bag at 2 - 25°C.

Opened aluminium bag: Use test cassette within 8 hours!

LIODetect®TB-ST Diluent (dilution buffer): Store the LIODetect®TB-ST Diluent at 2 - 25°C. Unopened LIODetect®TB-ST Diluent is stable until the expiry date. After first opening the LIODetect®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 labels).
- Do not use reagents from different kit lots or batch codes and avoid mixing of reagents of different kit lots or batch codes.
- Before use bring all reagents to room temperature (preferably 15 - 25°C)!
- Only for serum, plasma or whole blood. Do not use the test with other body fluids.
- Avoid contamination of the reagents. Do not use the same container for several samples! Use separate single-use pipets 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 because 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 the contact of kit reagents with skin, eye or mucosa.
- All kit components should be considered as infectious agents. Decontaminate 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 kit reagents and samples to skin, eye and mucous. Avoid touching of the membrane in the result window of the test device with your fingers (danger of contamination).
- Do not pipette samples and LIODetect®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 **LIODetect®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 leaked 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. For 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 LIODetect®TB-ST Diluent** into the sample well (S).
4. Start stop watch after addition of LIODetect®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 LIODetect®TB-ST Diluent** into the sample well (S).
4. Start stop watch after addition of LIODetect®TB-ST Diluent and read the results after 20 minutes.

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DIN EN ISO 13485

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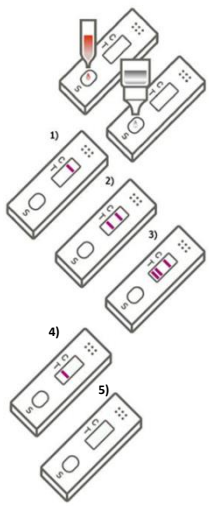
QUICK REFERENCE GUIDE



Serum / plasma:
2 drops of sample
Whole blood:
1 drop of sample

2 – 3 drops of
TB-ST Diluent

20 min

Results:
1 = NEGATIVE (Control line only appears)
2 = POSITIVE (2 lines)
3 = POSITIVE (3 lines)
Invalid:
4 = only test line(s) appears, no control line
5 = neither the control nor the test(s) line appears

INTERPRETATION OF RESULTS

NEGATIVE: Only one colored line appears in the control zone (control line "C", see Quick Reference Guide, and Fig. 1). In the test zone ("T") there should be no line visible.

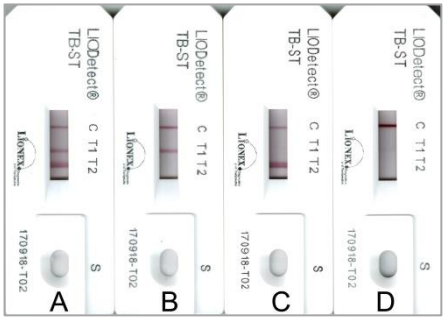
POSITIVE: Two or three colored lines appear. One line should be visible in the control zone ("C") and one or two other lines in the test zone ("T") (see Quick Reference Guide, and Fig. 1).

The test lines "T" may be stronger or weaker than the control line "C".

DOUBTFUL: Very weak shadow like test line(s) should be regarded as not clear. In this case it is recommended to take another sample from the same patient after 2-4 weeks and to measure it again using the **LIODetect®TB-ST Tuberculosis Rapid Test**. Furthermore, it is recommended to perform an interferon gamma release assay (IGRA) with the patient blood to confirm or exclude a possible infection.

Fig. 1: Examples of possible test results:

Positive test results (A) - (C): one or two test lines appear; Negative test result (D): only the control line appears.



INVALID: No control line visible and / or background color affects readability of test results.

Insufficient sample volume or incorrect handling of the test are the most likely reasons for a lack of control line and / or a formation of background color which affects the readablity of control / test lines. Check again the instructions of sample preparation and test procedure and repeat the test with a new test device. If the problem persists, contact the manufacturer or your local distributor.

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).

Method		Clinical diagnosis	
		TB	No TB
LIODetect® TB-ST	negative	53	312
	positive	100	11
Total results		153	323
% Concordance		**65.36**	**96.59**

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.

Method		Clinical diagnosis	
		TB	No TB
LIODetect® TB-ST	negative	25	94
	positive	67	6
Total results		92	100
% Concordance		**72.83**	**94.00**

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 (LTBI) 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:

Substance	Testing concentration
Acetaminophen	20 mg/dL
Acetylsalicylic acid	20 mg/dL
Ascorbic acid	20 mg/dL
Bilirubin	60 mg/dL
Caffeine	20 mg/dL
EDTA	20 mg/dL
Glucose	2000 mg/dL
Haemoglobin	500 mg/dL
Tetracycline	20 mg/dL
Sodium heparin	3 mg/dL
Lithium heparin	3 mg/dL

No interference is observed for one of the substances tested.

LITERATURE

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	Please consult instructions for use	REF	Cataloge number		Consumables: use by... (expiry date)		Protect from moisture
	Manufacturer		Do not reuse		Do not use if damaged	CE	Confirms to IVD-Directive 98/79/EG
2°C 25°C	Store at 2 - 25°C	LOT	Batch code	Σ	Contains sufficient for <n> tests	IVD	For in-vitro diagnostic use only