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. For performance evaluation only.

REF: 3010/3020/3050 **Rev. Draft**

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 or plasma within 20 minutes.

This test is intended for research use only and should not be used for diagnostic purposes.

INTRODUCTION / FIELD OF APPLICATION

Human tuberculosis (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*ycobacterium 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 to *M. tuberculosis* within 20 minutes.

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 *Mycobacterium tuberculosis* in serum or plasma.

The test consists of one test strip, which is integrated in a test cassette. This test strip consists of the following components:

1) A special antibody-binding protein, coupled to colloidal gold particles (conjugate).
2) A membrane with two test lines and one control line.

One test line contains a unique combination of TB antigens, the other one consists of a single cell wall antigen. The control line consists of an antibody-binding protein.

After the sample (serum or plasma) is pipetted followed by the LIODetect®TB-ST Diluent into the sample well (S), the diluted sample passes through the gold-labelled antibody-binding protein (conjugate). The immunoglobulins 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 / cell wall antigen are immobilized (test lines). If antibodies against tuberculosis are present in the sample, these bind to the test line / s.
In the result window of the test device, one or two pink / purple lines appear in the **test zone ("T")**. The remaining complex migrates further across the membrane to the **control zone ("C")**. Again a pink / purple line appears, indicating that the test was performed correctly.

SUPPLIED MATERIALS

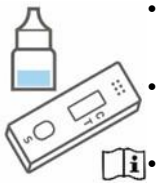
Packaging sizes:

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

REF: 3020 (20 Tests): 20 test cassettes and 2 dropper bottle containing 3.5 mL LIODetect®TB-ST Diluent.

REF: 3050 (50 Tests): 50 test cassettes and 5 dropper bottle containing 3.5 mL 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 - 30°C. Unopened kit components (aluminum bags and LIODetect®TB-ST Diluent) are stable until the expiry date. The expiry date is printed on the labels of the aluminum pouch, 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.

Aluminum pouch with test cassette: Keep the test in unopened aluminum bag at 2 - 30°C.

Opened aluminum bag: Use test cassette within one day!

LIODetect®TB-ST Diluent (dilution buffer): Store the diluent at 2 - 30°C. Unopened diluent is stable until the expiry date. After first opening the 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) or ISO9001, 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 - 30°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 *research* 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 (test cassettes and LIODetect®TB-ST Diluent) and samples in accordance to local regulations, e.g. by autoclaving or using a disinfecting solution.
- 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 one day.

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 lay down.
- 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 the single use capillary. Try to touch only the leaked blood and avoid air bubbles.

Serum or plasma: 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 3 days 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:

1. Take the required number of test cassettes from the packaging kit. Remove the aluminum pouch 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 20 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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EN ISO 9001:2008
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INTERPRETATION OF RESULTS

NEGATIVE: Only one pink / purple 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.

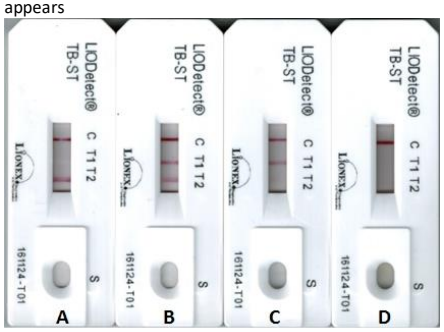
POSITIVE: Two or three pink / purple lines appear. One line should be visible in the control zone ("C") and one or two other lines in the test zone ("T") (Fig. 1).

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

NOT CLEAR: Very weak shadow like test lines should be regarded as negative. 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 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 colour affects readability.

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 colour which affects the readablity of control / test lines. Check again the instructions of sample preparation / 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 pink / purple 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 colour 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, 557 samples were evaluated with the **LIODetect®TB-ST Tuberculosis Rapid Test**.

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 was defined (positive control group). As "negative control", samples of clinically healthy donors and patients with other diseases were measured. Overall, 355 negative samples (possible latent TB, BCG vaccinated, other diseases and healthy subjects) and 92 samples of TB patients were measured (culture positive). The specificity was 95% with a sensitivity of 91.3%.

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 tuberculosis (TB)**. The test is not suitable for the detection of so-called latent TB (LTBI) or for contact screening of populations. Infections with other pathogenic mycobacteria cannot be detected with this test.

The test must not be used for diagnostic purposes. 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. If the result is **not clear** it is recommended to perform an interferon gamma release assay (IGRA) with the patient to confirm or exclude a possible infection.

NOTE: If precipitates appear in the LIODetect®TB-ST Diluent, this will not affect the test results!

Interfering substances:

An excessive amount of lipids in a sample may cause due to inhomogeneity physicochemical interferences. In addition, high concentration of lipids can potentially change the binding behavior of antibodies and thus falsify the results. Therefore, lipaemic samples may not be used.

Haemolysed samples should not be used because certain components of red blood cells pass into plasma or serum and might have potentially affects onto test results.

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.

LITERATURE

Al Zahrani K, Al Jahdali H, Poirier L, Rene P, Gennaro ML, Menzies D. Accuracy and utility of commercially available amplification and serologic tests for the diagnosis of minimal pulmonary tuberculosis. Am J Respir Crit Care Med. 2000 Oct; 162 (4 Pt 1): 1323-9.

Baumann R, Kaempfer S, Chegou NN, Nene NF, Veenstra H, Spallek R, Bolliger CT, Lukey PT, van Helden PD, Singh M, Walzl G. Serodiagnostic markers for the prediction of the outcome of intensive phase tuberculosis therapy. Tuberculosis (Edinb). 2013 Mar;93(2):239-45. doi: 10.1016/j.tube.2012.09.003. Epub 2012 Nov 2.

Baumann, R; Kaempfer, S; Chegou, N N.; Oehlmann, W; Spallek, R; Loxton, A G.; van Helden, P D.; Black, Gillian F.; Singh, M; Walzl, G. 2015. A Subgroup of Latently Mycobacterium tuberculosis Infected Individuals Is Characterized by Consistently Elevated IgA Responses to Several Mycobacterial Antigens. Mediators of Inflammation . Vol. 2015, p1-10. 10p

Delph,i Chatterjee and Kay-Hooi Khoo: Mycobacterial lipoarabinomannan: An extraordinary lipoheteroglycan with profound physiological effects. Glycobiology. 1998 Feb; 8(2):113-20.

Friscia G, Vordermeier HM, Pasvol G, Harris DP, Moreno C, Ivanyi: Human T cell responses to peptide epitopes of the 16-kD antigen in tuberculosis.J. Clin Exp Immunol 1995 Oct; 102 (1): 53-7.Verbon A,

Gennaro ML. Immunologic diagnosis of tuberculosis. Clin Infect Dis. 2000 Jun; 30 Suppl 3: 243-6. Review.

Imaz MS, Comini MA, Zerbini E, Sequeira MD, Spoletti MJ, Etchart AA, Pagano HJ, Bonifasich E, Diaz N, Claus JD, Singh M. Evaluation of the diagnostic value of measuring IgG, IgM and IgA antibodies to the recombinant 16-kilodalton antigen of mycobacterium tuberculosis in childhood tuberculosis. Int J Tuberc Lung Dis 2001 5 (11): 1036-43

Sardellal I G; Mahavir S; Kumpfer S; Ribeiro Heringer R ; Féres Saad M H, +; Puccioni Sohler M: Evaluation of Lionex TB kits and mycobacterial antigens for IgG and IgA detection in cerebrospinal fluid from tuberculosis meningitis patients. Print version ISSN 0074-0276, Mem.

Kaisermann MC, Sardella IG, Trajman A, Coelho LV, Kämpfer S, Jonas F, Singh M, Saad MH.: IgA antibody responses to Mycobacterium tuberculosis recombinant MPT-64 and MT-10.3 (Rv3019c) antigens in pleural fluid of patients with tuberculous pleurisy. Int J Tuberc Lung Dis. 2005 Apr;9(4):461-6.PMID: 15830754

Khoo, K. H., Tang, J. B., and Chatterjee, D.: Variation in Mannose-capped Terminal Arabinan Motifs of Lipoarabinomannans from Clinical Isolates of Mycobacterium tuberculosis and Mycobacterium avium Complex (2001) J. Biol. Chem. 276, 3863-3871

Kuijper S, Jansen HM, Speelman P, Kolk AH: Development of a serological test for tuberculosis Ned Tijdschr Geneeskd 1991 Jan 26; 135 (4): 134-8

Lenka M. Pereira Arias-Bouda, Lan N. Nguyen, Ly M. Ho, Sjoukje Kuijper, Henk M. Jansen, and Arend H. J. Kolk: Development of Antigen Detection Assay for Diagnosis of Tuberculosis Using Sputum Samples Journal of Clinical Microbiology, June 2000, p. 2278-2283, Vol.38, No.6 0095-1137/00/ $04.00+0

Lyashchenko KP, Singh M, Colangeli R, Gennaro ML. A multi-antigen print immunoassay for the development of serological diagnosis of infectious diseases. J Immunol Methods. 2000 Aug 28; 242 (1-2): 91-100.

MURAMIRA M. Nobert, Dr OLAF MÜLLER, Dr. MUSEMAKWERI André: EVALUATION OF TB-ST TUBERCULOSIS RAPID TEST IN ADULT PULMONARY TUBERCULOSIS IN RWANDA. Huye, Universite Nationale du Rwanda, FACULTY OF MEDICINE October 2007.

Pant Pai, N and Pai M: Point-of-Care Diagnostics for HIV and Tuberculosis: Landscape, Pipeline, and unmet needs. Discovery Medicine, Volume 13, Number 68 p.35-45. Jan. 2012. ISSN: 1539-6509

Pukazhvanthen P, Anbarasu D, Ahamed Kabeer Basirudeen S, Raja A, *, Singh M: Assessing humoral immune response of 4 recombinant antigens for serodiagnosis of tuberculosis. Tuberculosis 94 (2014) 622e633.

Samanich KM, Keen MA, Vissa VD, Harder JD, Spencer JS, Belisle JT, Zolla-Pazner S, Laal S. Serodiagnostic potential of culture filtrate antigens of Mycobacterium tuberculosis.Clin Diagn Lab Immunol. 2000 Jul; 7(4): 662-8.

Samanich K, Belisle JT, Laal S. Homogeneity of antibody responses in tuberculosis patients. Infect Immun. 2001 Jul; 69 (7): 4600-9.

Silva VM, Kanaujia G, Gennaro ML, Menzies D. Factors associated with humoral response to ESAT-6, 38 kDa and 14 kDa in patients with a spectrum of tuberculosis. Int J Tuberc Lung Dis. 2003 May; 7 (5): 478-84.

Van Deun A, Portaels, F: Limitations and requirements for quality control of sputum smear microscopy for acid-fast bacilli. J Clin Microbiol 2000 Jun;38(6):2278-83.

Verbon, A; Kuijper, S; Jansen, H M; Speelman, P; Kolk, A H J. Antigens in culture supernatant of M. tuberculosis: epitopes defined by monoclonal and human antibodies. J Gen Microbiol. 1990;136:955–964

Wilkinson, R. J., K. Hasløv, R. Rappuoli, F. Giovannoni, P. R. Narayanan, C. R. Desai, M. Vordermeier, J. Paulsen, G. Pasvol, J. Ivanyi, and M. Singh. Evaluation of the recombinant 38-kilodalton antigen of Mycobacterium tuberculosis as a potential immunodiagnostic reagent. J. Clin. Microbiol. 1997. 35: 553-557

WHO Tuberculosis Diagnostics Workshop: – Product Development Guidelines – Cleveland, Ohio, 27 July, 1997.

Please consult instructions for use		Consumables: use by... (expiry date)		Protect from moisture	
Manufacturer	Do not reuse	Do not use if damaged		For performance evaluation only	
Store at 2 - 30°C	Batch code	Contains sufficient for <n> tests		Cataloge number	