LIODetect®TB Urine
Tuberculosis Rapid Test

Qualitative rapid test for the detection of cell wall antigens from *Mycobacterium tuberculosis* in Human Urine. For professional use. For research use only.

INTENDED USE

The **LIODetect®TB Urine Tuberculosis Rapid Test** is an In-vitro diagnostic rapid test for the qualitative detection of cell wall antigen from *Mycobacterium tuberculosis* in human urine within 20 minutes.

The test is for research use only. It must not be used for diagnosis.

INTRODUCTION / FIELD OF APPLICATION

M. tuberculosis is the causative agent of human tuberculosis (TB), which is primarily an illness of the respiratory system, and is spread by coughing and sneezing. People suffering from active pulmonary TB are highly infectious. TB has become a global disease with its re-emergence in the Western countries in the last decades. Overall, more than 30 % of the world's population is estimated to be infected with the TB bacillus what kills yearly about 2 million people, more than any other infectious disease.

Active TB is highly infectious and easily spread by coughing and sneezing. Latent TB infection (LTBI) is not infectious and occurs when the individual's for life and can reactivate when the individual's immune system is weakened, for example, at old age or due to infection from other diseases.

Symptoms of TB vary according to the site of infection. These include poor general health, weight loss, fatigue and weakness.

Symptoms of Pulmonary TB include fever, chest pain, night sweats, persistent coughing and blood-stained sputum. Primary TB can be asymptomatic and active disease may only occur in 10% of infected individuals. Diagnosing TB can be difficult and complex, a selection of diagnostic techniques is necessary in order to diagnose the disease correctly. The **LIODetect®TB Urine Tuberculosis Rapid Test** is a cost effective and rapid lateral flow based assay which detects cell wall antigen from mycobacteria. The test can detect the antigen in urine in two steps within app. 20 minutes.

PRINCIPLE OF THE TEST

LIODetect®TB Urine Tuberculosis Rapid Test is a membrane-based test for the rapid detection of cell wall antigen in urine. This innovative and sandwich type rapid test is based on lateral flow immune-chromatography.

After the sample is pipetted into the sample well together with the diluent buffer it passes through the gold-marked protein (conjugate). If any antigen is present in the sample it attaches to the conjugate. This antigen-conjugate complex then flows through the membrane to the point where the capture antibodies to MTB antigens are immobilized (test line) resulting into a red band. The remaining conjugate complex then passes through the membrane until it reaches the control zone. Again, a red band appears, indicating that the test has been performed properly.

KIT CONTENTS

Packaging sizes:

REF   AG004_10_EN (10 Tests):

10 test cassettes, one dropper vial with 3.5 mL LIODetect®TB Urine Diluent.

TEST COMPONENTS

- LIODetect®TB Urine 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 PROVIDED

- Stop watch
- Containers for sample collection. We recommend using standard containers for urine collection.
- Incubator for heat inactivation (T = 100°C) or autoclave.
- Rack for placing the vials.

PREPARATION OF REAGENTS

All reagents are ready to use.

STABILITY AND STORAGE

Store at 2 - 30°C. Unopened tests (container with LIODetect®TB Urine tests and Diluent) are stable until expiry date. Expiry date of tests and diluent are printed on the labels of the outer package. Do not use, if the vial or pouches are damaged!

DO NOT FREEZE or expose to temperatures above 30°C for longer time.

Tests in unopened pouch and diluent: store at 2 - 30°C.

Opened pouch: Use tests on the same day.

PRECAUTIONS AND WARNINGS

- 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 (mentioned on the kit label). After removing from the container, the test strip must be used at the same day.
- Do not use reagents from different kit lots and do not mix reagents of different kits or kit lots with one another.
- Before use, bring all reagents to room temperature (15 - 30°C)!
- For urine and only. Not for other body fluids.
- Avoid contamination of the reagents. Do not use the same container for several samples!
- Do not use bacterial contaminated samples.
- Avoid repeated freezing and thawing of samples, because the antigen may be destroyed.

- The test is 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 and lab coat)! Avoid the contact of kit reagents with skin, eye or mucosa. After test performance, clean hands carefully.
- Avoid spilling reagents and Aerosol formation.
- Wipe-off spilled reagents and samples at once and disinfect.
- Urine samples may be infectious. Take care for safe handling and disposing.
- All kit components should be considered as infectious agents. Wipe-off sample and reagent spills with a disinfecting solution (e.g. sodium hypochlorite, 5%)! Dispose residues of kit reagents and samples properly, e.g. by autoclaving.
- For single use only. Do not use, if the vials with liquids are damaged. Close the vials tightly after removing liquids.
- For research use only. Not for diagnosis!

SAMPLE PREPARATION AND COLLECTING THE SAMPLES

LIODetect®TB Urine Tuberculosis Rapid Test is designed to detect cell wall antigen from *Mycobacterium tuberculosis* in human urine within 20 minutes.

The test works best with fresh samples.

Collecting and preparing the samples:
General information:
- Samples shall be collected in clean and heat stable plastic containers.

Collecting the urine:
- Collect midstream clean catch specimen of urine in an appropriate container.
- Close the container and test the sample as soon as possible (within 1 h).
- If testing is not possible within 1 hour, keep the sample at T < -20°C.

Preparation of the urine samples:
We recommend autoclaving (121°C / 20 min) or boiling (e.g. for 10 min at 100°C in a thermo block).

Storage of samples:
The samples can be stored refrigerated (2 - 8 °C) for up to 1 hour. For a longer storage, keep samples below -20 °C.

TEST PROCEDURE

1) Pipet 60 µL (or 2 drops using the pipet for single use) of the sample into the sample well (SHORT REFERENCE GUIDE 1)

2) Add 3 drops of diluent buffer into the sample well (SHORT REFERENCE GUIDE 2A).

3) Adjust timer to 20 min. (SHORT REFERENCE GUIDE 2B).

4) Read the results after 20 min (SHORT REFERENCE GUIDE 3).
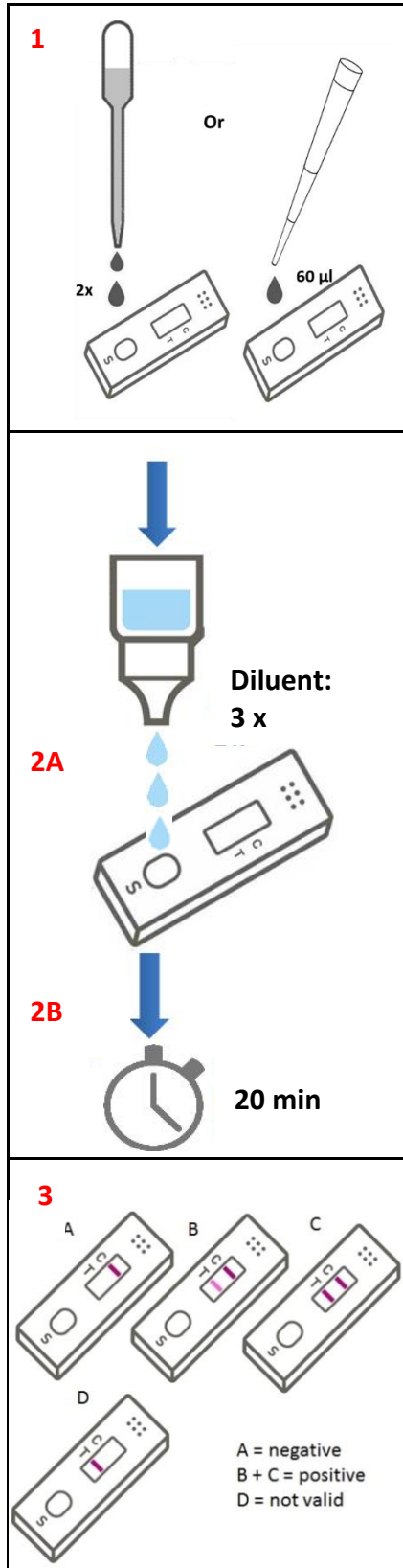
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REF: AG004_10_EN   Rev. 1.0   /200122

SHORT REFERENCE GUIDE

1

Or

2x   60 µl

Diluent:
3 x

2A

2B

20 min

3

A = negative
B + C = positive
D = not valid

INTERPRETATION OF RESULTS

NEGATIV: One pink / purple line appears (control line „C"). No further line is visible.

POSITIVE: Two pink-purple bands appear; one in the control area and the other in the test area. High concentration samples yield results within 20 minutes.

NOT VALID: No line appears or in the control area („C") or no line appears on the membrane.

Intensity of the test line may be stronger or weaker. Each distinct test line is a positive result, regardless on its intensity.

Appearance of a very faint shadow-like line:

Indeterminate results: In this case, take a new sample from the same patient and repeat testing.

Most likely reason for absence of the control line is insufficient sample volume or mishandling. Check your protocol and repeat testing by using a new test strip. If the problem persists, contact your local distributor.

QUALITY CONTROL

The **LIODetect®TB Urine Tuberculosis Rapid Test** contains an internal control. The pink / purple line appearing in the control area („C") indicates that the test is used properly. The control line confirms sufficient sample volume and correct performance. A clear background on the membrane is the internal negative control.

PERFORMANCE CHARACTERISTICS

For detecting analytical sensitivity, urine was spiked by defined concentrations of cell wall antigen (urine pool, heat inactivated). The spiked samples are measured by **LIODetect®TB Urine Tuberculosis Rapid Test**. The detection limit was below 100 ng/ml. Using special filters for urine, the detection limit can be reduced to less than 50 ng/ml.

Our antibody conjugate is highly specific to "*M tuberculosis* COMPLEX" cell wall antigen. No cross reaction was observed with *M. avium* and *M intracellulare* cultivated on Sauton's medium.

LIMITATIONS

LIODetect®TB Urine Tuberculosis Rapid Test has been developed to detect cell wall antigen from *M. tuberculosis* in human urine. Using this test for other fluids has not yet been validated and can yield incorrect results.

The test is specific for Lipoarabinomannan (LAM) from *M. tuberculosis* Complex. Cross-reactions with other pathogenic mycobacteria such as *M. avium* and *M intracellulare* have not been observed.

The test must not be used for diagnostic purposes.

REFERENCES

Brennan,P.J. and Nikaido,H. (1995) The envelope of mycobacteria. Annu. Rev.Biochem., 64, 29–63.

Delphi Chatterjee and Kay-Hooi Khoo: Mycobacterial lipoarabinomannan: An extraordinary lipoheteroglycan with profound physiological effectsGlycobiology. 1998 Feb; 8(2):113-20.

Harshini Mukundana, Sandeep Kumara, Dominique N. Pricea, Sonja M. Rayb, Ye-Jin Leec, Seonyeong Minc, Seokyong Eumc, Jessica Kubicek-Sutherlanda, Jesse M. Resnicka, W. Kevin Gracea, Aaron S. Andersona, Soo Hee Hwangd, Sang Nae Choc, e, Laura E. Viab, Clifton Barry IIIb, Ramamurthy Sakamuria, Basil I. SwansonaRapid detection of Mycobacterium tuberculosis biomarkers in a sandwich immunoassay format using a waveg<uide-based optical biosensor. Tuberculosis; Volume 92, Issue 5, September 2012, Pages 407–416.

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

Stephen D Lawn: Point-of-care detection of lipoarabinomannan (LAM) in urine for diagnosis of HIV-associated tuberculosis: a state of the art review. BMC Infectious Diseases 2012, 12:103

Lawn SD, Andrew D Kerkhoff, Monica Vogt, and Robin Wood: Diagnostic accuracy of a low-cost, urine antigen, point-of-care screening assay for HIV-associated pulmonary tuberculosis before antiretroviral therapy: a descriptive study. Lancet Infect Dis. 2012 Mar; 12(3): 201–209. Doi: 10.1016/S1473-3099(11)70251-1. PMCID: PMC3315025

Lawn SD: Point-of-care detection of lipoarabinomannan (LAM) in urine for diagnosis of HIV-associated tuberculosis: a state of the art review. BMC Infect Dis. 2012 Apr 26; 12:103. Doi: 10.1186/1471-2334-12-103.

Lawn SD, Edwards DJ, Kranzer K, Vogt M, Bekker LG, Wood R.: Urine lipoarabinomannan assay for tuberculosis screening before antiretroviral therapy diagnostic yield and association with immune reconstitution disease. AIDS. 2009 Sep 10;23 (14):1875-80.

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

Pant Pai, Nitika and Madhukar Pai: 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

Tessema TA, Hamasur B, Bjun G, Svenson S, Bjorvatn B: Diagnostic evaluation of urinary lipoarabinomannan at an Ethiopian tuberculosis centre. Scand J Infect Dis. 2001;33(4):279-84.

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

Yáñez, M A; Coppola, M P; Russo, D A; Delaha, E; Chaparas, S D; Yeager, H., Determination of mycobacterial antigens in sputum by enzyme immunoassay. J Clin Microbiol. 1986;23:822–825.

📖 Please consult instructions for use	⌛ Consumables: use by … (expiry date)	
🏭 Manufacturer	⊘ Do not reuse	⊗ Do not use if the outer package is damaged	For research use only.
🌡 30°C Store at 2 - 30°C	LOT Batch code	Σ Contains sufficient for <n> tests / determinations	REF Catalogue number