TUB IgA ELISA HUMAN TUBERCULOSIS TEST

Microtiter plate based Enzyme assay for the quantitative detection of human IgA antibodies to *Mycobacterium tuberculosis* in serum or plasma. For research use only.

REF: LIO-TUB02 **Rev. 1.0 / 221104**

INTENDED USE

The **TUB IgA ELISA** is an microtiter plate based Enzyme assay for the quantitative detection of human IgA antibodies to *Mycobacterium tuberculosis* in serum or plasma within 100 minutes.

This test is intended for research use only.

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. 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 time consuming. The diagnosis of TB is usually made based on a combination of several laboratory tests.

The **TUB IgA ELISA** is suitable for the quantitative detection of human IgA antibodies to *Mycobacterium tuberculosis* within 100 minutes.

PRINCIPLE OF THE TEST

The **TUB IgA ELISA** is based on the principle of the enzyme immunoassay (EIA) and is intended for the quantitative detection of human IgA antibodies to *Mycobacterium tuberculosis* in serum or plasma within 100 minutes.

Highly purified specific antigens are bound on the surface of the microtiter plate. Diluted serum or plasma from human are pipetted into the wells of the microtiter plate at once with the ready-to-use standard solutions. A binding between the antibodies of the samples and the immobilized antigens takes place. After 45 minute incubation at 37°C, the plate is rinsed with diluted wash buffer, in order to remove unbound material. Then conjugate solution (peroxidase-conjugated anti-human antibody) is added into the wells and incubated for 30 minutes at 37°C. In this step, the conjugate binds to the antigen-specific antibodies from the samples (if available) and standard solutions. After a further washing step, the substrate solution for the peroxidase is pipetted into the wells and incubated for 20 minutes at 37°C, inducing the development of a blue dye in the wells. The intensity of this reaction is dependent on the amount of specific antibodies in the samples. The colour development is terminated by the addition of a stop-solution, which changes the colour from blue to yellow. The resulting dye is measured by an ELISA reader at the wavelength of 450 nm (optionally 620 nm). The concentration of specific antibodies is directly proportional to the intensity of the colour.

SUPPLIED MATERIALS

Packaging sizes:

REF LIO-TUB02 (43 Tests):

1 microtiter plate (96 wells) with 12 x 8 breakable strips suitable for 43 samples (duplicate measurements), including liquids: sample diluent, wash buffer, 4 standard solutions, conjugate solution, substrate solution and stop-solution.

TEST COMPONENTS

	PL	Microtiter plate (96 wells), ready-to-use, coated with *Mycobacterium tuberculosis* antigens (12 x 8 individual breakable strips) sealed in an aluminum pouch with a desiccant bag.
	PV	Sample Diluent: 100 mL, ready-to-use, in PE-vial, PBS/BSA buffer, contains 0.05% 5-bromo-5-nitro-1.3-dioxane.
	WP	Wash Buffer, 10 x concentrate: 60 mL, in PE-vial, PBS buffer with Tween 20, contains 0.05% 5-bromo-5-nitro-1.3-dioxane.
1 2 3 4	STA	4 standard solutions: 2 mL each, ready-to-use, in PE-vials, with different amounts of relevant specific antibodies against *Mycobacterium tuberculosis*, contains 0.05% 5-bromo-5-nitro-1.3-dioxane.
	CON	Anti-human-IgA-conjugate solution: 12 mL, ready-to-use, in PE-vial, contains 0.05% 5-bromo-5-nitro-1.3-dioxane.
	TMB	TMB substrate solution: 12 mL, ready-to-use, in PE-vial (black), contains a solution of tetramethylbenzidine (TMB).
	STO	Stop-solution: 12 mL, ready-to-use, in PE-vial, 0.2 M H_2SO_4.
		1 instructions for use.
		Plastic bag: Reseal-able, for the dry storage of unused microtiter plate/strips.

MATERIALS NEEDED BUT NOT SUPPLIED

- Stop watch.
- Containers for sample collection. We recommend using standard containers for blood collection.
- Microliter pipets and tips.
- ELISA Reader with filter (450 nm or optionally 620 nm).
- Automatic washer for microtiter plates (optional).
- Vials for sample preparation.
- Measuring cylinder for wash buffer preparation.
- De-ionized or distilled water.
- Incubator (37°C).

PREPARATION OF REAGENTS

WP Wash Buffer: Dilute 1:10 with de-ionized or distilled water before use (1 volume wash buffer + 9 volumes water). If crystals precipitate during the cold storage, the concentrate solution should be warmed up at 37°C for 15 minutes.

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

STABILITY AND STORAGE CONDITIONS

Store the test at 2 - 8°C. Unopened kit components are stable until the expiry date. The expiry date is printed on the labels of the aluminum pouch (microtiter plate), on the labels of the containers for the liquids and on the outer packaging.

Do not use if the aluminum pouch is damaged. **DO NOT FREEZE** or expose temperatures above 30°C (except wash buffer, if crystal precipitate occurs).

Aluminum pouch with microtiter plate: Keep the test in unopened aluminum bag at 2 - 8°C.

Opened aluminum pouch with microtiter plate: Use up microtiter plate within 6 months!

Liquids: Keep liquid components at 2 - 8°C. Unopened liquids are stable until the expiry date. After first opening the liquids are stable for 6 months, if the bottles are tightly closed after every usage.

WARNINGS AND PRECAUTIONS

- **ATTENTION:** Avoid contact of skin, eye and mucosa to the STO stop-solution. Causes severe skin burns and eye damage! Wear safety glasses, gloves and protective clothing!
- **ATTENTION:** Avoid contact of skin, eye and mucosa to the TMB substrate solution. The solution may damage fertility or the unborn child! Wear safety glasses, gloves and protective clothing!
- 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 15-30°C)!
- Only for human serum or plasma. Do not use the test with other body fluids.
- Avoid contamination of the reagents. Do not use the same container for several samples!
- 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 protective clothing)! 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 contents and samples in accordance to local regulations, e.g. by autoclaving or using a disinfecting solution.
- Avoid contamination of the reagents by using separate disposable pipet tips. Close bottles tightly immediately after removing reagents.
- Before pipetting, mix all reagents thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- Pipet with constant intervals, so that all wells of the microtiter plate have the same conditions!
- For single use only. Do not use microtiter plate if the outer packaging (aluminum pouch) is damaged. After opening the pouch, the microtiter plate/strips must be used within 6 months. After opening store microtiter plate/strips in the plastic bag provided together with the desiccant bag.

SAMPLE COLLECTION AND PREPARATION

The **TUB IgA ELISA** test works best with fresh samples.

Collection of whole blood from the vein: Take the sample under standard laboratory conditions (aseptically, avoid haemolysis).

Serum or plasma: Separate the red blood cells as soon as possible (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!

Manufacturer / Distributor:
LIONEX GmbH
Salzdahlumer Str. 196, Geb. 1A
38126 Braunschweig / Germany

Phone: + 49 (0) 531 - 260 12 66
Fax: + 49 (0) 531 - 618 06 54
E-mail: info@lionex.de
Homepage: www.lionex.de

TUB IgA ELISA HUMAN TUBERCULOSIS TEST

Microtiter plate based Enzyme assay for the quantitative detection of human IgA antibodies to *Mycobacterium tuberculosis* in serum or plasma. For research use only.

LIONEX Diagnostics and Therapeutics

REF: LIO-TUB02 **Rev. 1.0 / 221104**

Sample preparation (serum or plasma): For the performance of the test, the samples (not the standard solutions) have to be diluted 1:200 with **PV** sample diluent (e.g. 5 µL sample + 995 µL sample diluent)!

ATTENTION: All standard solutions are ready-to-use and MUST NOT be diluted!

TEST PROCEDURE

Test procedure time is 100 minutes:

1. Preparation of reagents:

   Equilibrate all kit components to room temperature (preferable 15 - 30°C). Dilute 10 x concentrated **WP** wash buffer 1:10 with de-ionized or distilled water (1 + 9 volume). Dilute serum or plasma samples 1:200 with **PV** sample diluent (e.g. 5 µL sample + 995 µL sample diluent)!

2. STEP A: Pipet 100 µL of 1:200 diluted samples, each ready-to-use **STA** standard solution and **PV** sample diluent for blank value into the wells (all in duplicates).

   INCUBATION: 45 minutes at 37°C.

3. STEP B: Washing procedure: Empty the wells of the plate (dump or aspirate) and wash 3 x 300 µL per well with 1:10 diluted **WP** wash buffer.

   Pipet 100 µL of **CON** conjugate solution into all wells.

   INCUBATION: 30 minutes at 37°C.

4. STEP C: Repeat washing procedure (3 x 300 µL of 1:10 diluted **WP** wash buffer per well).

   Pipet rapidly 100 µL of the **TMB** substrate solution into all wells.

   INCUBATION: 20 minutes at 37°C in the dark.

5. STEP D: Terminate the substrate reaction: Pipet rapidly 100 µL of the **STO** stop-solution into all wells.

6. Measuring procedure: Measure the absorption at 450 nm (optional reference wavelength: 620 nm). The colour is stable for at least 60 minutes.

QUICK REFERENCE GUIDE OF TEST PROCEDURE

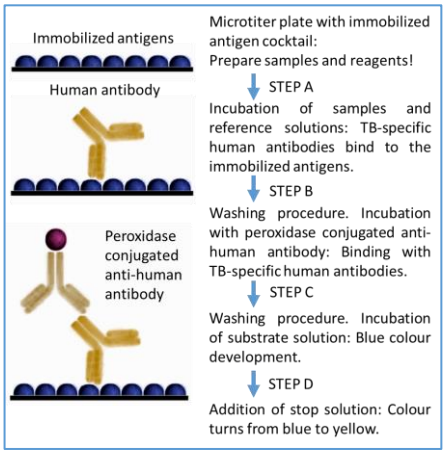


Fig. 1: Schematic presentation of test procedure of **TUB IgA ELISA** (REF: LIO-TUB02).

INTERPRETATION OF RESULTS

Calculate the **mean OD values** for the measured absorptions for every sample. Subtract the blank value from every calculated mean absorption. The difference between single values should not exceed 10% for the standard solutions and samples.

QUANTITATIVE RESULTS

The ready-to-use standard solutions of the Human Tuberculosis Test kits (TUB ELISA) are defined and expressed in relative Units (U/mL). This results in reproducible quantitative evaluation. Consequently, for a given patient, follow-up becomes possible. The values for standard solutions in units are printed on the labels of the vials. For interpretation of results, a generation of a scatter diagram with linear regression line is required (reference curve).

For calculation of the reference curve, we recommend to use automatic computer programs.

⇨ Plot the OD results of each standard solution on the horizontal axis (x = OD 450, optional OD 620 nm) against the number of corresponding units (the No. of units for each standard solution is printed on the label) on the vertical axis (y = U/mL).

In the scatter diagram with regression line, the relation between two variables is presented graphically and the regression line (the curve that fits best the plotted points) is drawn in the diagram. The equation of this curve is given in the regression window (figure 2). The resulting reference curve should be a straight line ($R^2 > 0{,}980$).

⇨ Calculate the concentration of each measured sample (in units) by using the equation of the reference curve:

U/mL = (b x mean OD value) + a

⇨ Multiply resulting concentration (U/mL) of every sample by 200 to calculate the level of serounits for each sample since the sample was diluted 1:200.

Serounits = 200 x (U/mL)

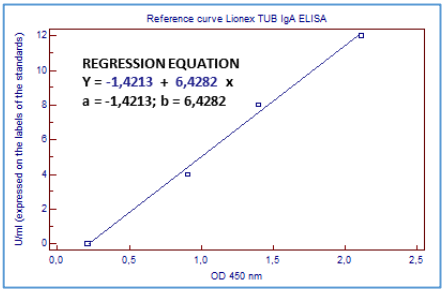


Fig. 2: Typical reference curve for TUB IgA ELISA (REF: LIO-TUB02, example).

The result of Human Tuberculosis Test kits (TUB ELISA) can be NEGATIV, BORDERLINE or POSITIVE.

ATTENTION: The test is for research use only!

CUT-OFF level

The CUT-OFF level of the **TUB IgA ELISA** is determined by the evaluation of several hundreds of samples from TB-patients and healthy individuals. But the CUT-OFF level may vary for patient populations from different regions and countries. Therefore, the CUT-OFF value should be determined individually by every new user. We recommend determining the CUT-OFF by measuring a panel of confirmed negative and positive cases (example is shown in figure 3).

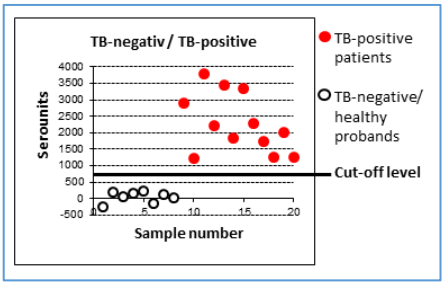


Fig. 3: Comparison of serounit values measured for TB-negative and TB-positive patient samples.

Based on our own results, we suggest the following CUT-OFF levels:

⇨ **Less than 200 serounits:** **NEGATIVE**
⇨ **More than 300 serounits:** **POSITIVE**
⇨ **In-between 200 - 300 serounits:** **BORDERLINE**

QUALITY CONTROL

The **TUB IgA ELISA** contains standard solutions as an internal control.

The colour of the liquid in the wells containing the standard solutions turn into blue after incubation of the substrate solution (20 minutes after addition of the substrate solution). After addition of the stop-solution the colour changes from blue to yellow. This colour development is considered an internal positive control. It confirms the correct procedural technique.

If the colour of the standard solutions do not turn into blue after incubation of the substrate solution, the test result is invalid.

A clear background in the wells containing the sample diluent is an internal negative control (blank). If a background colour (blue dye) appears in the negative control, the test result is invalid.

PERFORMANCE CHARACTERISTICS

Precision:

The intra-assay coefficient of variation (CV) of Human Tuberculosis Test kits (TUB ELISA) was determined by ten-fold repeated measurements of several positive samples. The intra-assay CV is less than 10%.

Sensitivity and specificity for serum/plasma:

To determine the sensitivity and specificity 985 serum and plasma samples from different regions were evaluated with the **TUB IgA ELISA**. The results of the **TUB IgA ELISA** were compared with clinical outcomes.

As gold standard a pathogen detection based on cultivation was defined (positive control group, a total number of 460 samples). As "negative control", samples of clinically healthy donors and patients with other diseases were measured (525 samples).

Extensive studies on samples of TB patients (culture positive) showed a sensitivity of 79.13% and a specificity of 96.95%. The cumulative sensitivity could be additionally increased by simultaneous measurement of IgG and IgA antibodies (figure 4).

The positive predictive value (PPV) was 0.9579 with a negative predictive value (NPV) of 0.8413.

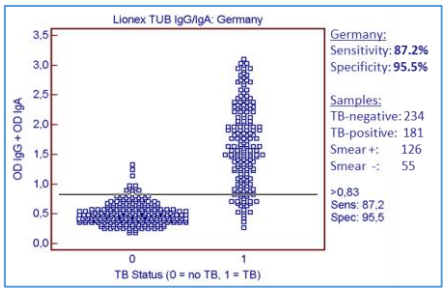


Fig. 4: Sensitivity and specificity of TUB IgG and IgA ELISA (cumulative); Diagram show results with samples from Germany.

Manufacturer / Distributor:
LIONEX GmbH
Salzdahlumer Str. 196, Geb. 1A
38126 Braunschweig / Germany

Phone: + 49 (0) 531 - 260 12 66
Fax: + 49 (0) 531 - 618 06 54
E-mail: info@lionex.de
Homepage: www.lionex.de

TUB IgA ELISA HUMAN TUBERCULOSIS TEST

Microtiter plate based Enzyme assay for the quantitative detection of human IgA antibodies to *Mycobacterium tuberculosis* in serum or plasma. For research use only.

REF: LIO-TUB02 **Rev. 1.0 / 221104**

LIMITATIONS

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

The **TUB IgA ELISA** is an microtiter plate based Enzyme assay for the quantitative detection of human IgA antibodies to *Mycobacterium tuberculosis* in serum or plasma within 100 minutes.

It is intended *for research use only*! For the measurement of other body fluids, this test has not been validated and results may be incorrect.

We recommend **measuring the same sample with all TUB ELISA kits** designed for detecting **IgG- (REF: LIO-TUB01), IgA- (REF: LIO-TUB02)**, and **IgM class antibodies (REF: LIO-TUB03)** to **increase the sensitivity**.

The test is *for research use only*, a definitive clinical diagnosis should be based on an evaluation of all clinical and laboratory findings by a doctor, and not by the results of the test. If a patient sample was tested as positive, more confirmatory tests must be performed.

POSITIVE test result needs further confirmation! For final diagnosis take all available clinical results of given patient into consideration (X-ray, microscopy, culture results, serology, clinical signs, PCR, clinical symptoms, IGRA-cytokine detection).

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 **BORDERLINE**, collect a fresh sample from the same patient after 2 - 4 weeks and repeat measurement. We recommend performing an Interferon Gamma Detection Test (IGRA), to confirm or exclude possible infection.

ATTENTION: The reference curve should be a straight line. OD values for ready-to-use standard solutions must be within the acceptable ranges defined in the kit inserts (± 10 %, valid range of COA - "Certificate of Analysis").

If the reference curve is not valid, CUT OFF level can be determined qualitatively as well by comparing the OD value of measured sample with the OD value of standard solution 2.

All samples with OD values below "OD standard solution 2" are NEGATIVE, samples with OD values above 1.2 x "OD standard solution 2" are POSITIVE.

NOT VALID: If, after 20 minute incubation with the substrate solution, the colour of the liquid in the wells containing the standard solutions does not turn into blue, the test result is invalid.

If, after 20 minute incubation with the substrate solution, a background colour (blue dye) appears in the wells containing the sample diluent (blank), the test result is invalid.

Insufficient sample preparation or sample volume or incorrect handling of the test (wrong sequence of steps during test performance) are the most likely reasons for invalid results. Check again the instructions of sample preparation / test procedure and repeat the test with new microtiter strips. If the problem persists, contact the manufacturer or your local distributor.

Interfering substances:

An excessive amount of lipids in a sample may cause physicochemical interferences due to inhomogeneity. 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 potential effects onto the test results.

Recent or ongoing treatment for TB may lead to faulty results. Antibody levels in the blood may diminish rapidly after treatment with anti-TB antibiotics and are maybe too low to detect by this test - 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.

Demkow U, Ziolkowski J, Bialas-Chromiec B, Filewska M, Zielonka T, Wasik M, Rowinska-Zakrzewska E. Humoral immune response against mycobacterial antigens in children with turbeculosis. J Physiol Pharmacol. 2006 Sep; 57 Suppl 4:63-73

Demkow U, Bialas-Chromiec B, Filewska M, Zielonka T, Michalowska-Mitczuk D, Kus J, Broniarek-Samson B, Augustynowicz-Kopec E, Zwolska Z, Rowinska-Zakrzewska E. Humoral immune response against mycobacterial antigens in patients with tuberculosis and mycobacterial infections other than tuberculosis. Pneumonol Alergol Pol. 2006; 74(2):203-8

Dolapo Awoniyi, Ralf Baumann, Novel Chegou, Belinda Kriel, Ruschca Jacobs, Martin Kidd, Andre Loxton, Susanne Kaempfer, Mahavir Singh, Gerhard Walzl. Combined specific IgG – and IgA – based diagnosis of Tuberculosis in African primary healthcare clinic attendees with signs and symptoms suggestive of Tuberculosis. DOI: 10.1136/bmjgh-2016-000260.94 Published 12 February 2017

Erer OF, Yalcin YA, Coskun M, Gündüz AT, Biçmen C, Ertas M, Ozkan SA. Humoral immune response against 38-kDa and 16-kDa mycobacterial antigens in tuberculosis. Senol G, Eur Respir J. 2007 Jan; 29(1):143-8. Epub 2006 Aug 9.PMID: 16899484

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 IgA, 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 IgA 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.

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.

Manufacturer / Distributor:
LIONEX GmbH
Salzdahlumer Str. 196, Geb. 1A
38126 Braunschweig / Germany

Phone: + 49 (0) 531 - 260 12 66
Fax: + 49 (0) 531 - 618 06 54
E-mail: info@lionex.de
Homepage: www.lionex.de

TUB IgA ELISA HUMAN TUBERCULOSIS TEST

Microtiter plate based Enzyme assay for the quantitative detection of human IgA antibodies to *Mycobacterium tuberculosis* in serum or plasma. For research use only.

LIONEX Diagnostics and Therapeutics

REF: LIO-TUB02 **Rev. 1.0 / 221104**

GIUDANCE OF TEST PROCEDURE

ATTENTION! Please follow the instructions for use carefully!

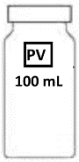
ATTENTION! Avoid contact of skin, eye and mucosa to the stop-solution and TMB-Substrate. The stop-solution is corrosive! The TMB-substrate may damage fertility or the unborn child! Wear safety goggles, gloves and protective clothing!

PREPARATION OF REAGENTS

Before use bring all reagents to room temperature (preferably 15 - 30°C)!

WP Wash buffer (green lid):

Dilute 1:10 with de-ionized or distilled water (1 + 9 volume). If crystals precipitate during the cold storage, the 10 x concentrate solution should be warmed up at 37°C for 15 minutes before dilution.



Sample preparation:

Store the serum, plasma or CSF samples at 2 - 8°C for up to 2 days (48 hours). For longer storage, the samples 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!

For the performance of the test, the samples have to be diluted **1:200** with **PV** sample diluent (white lid)! The standard solutions are ready-to-use and MUST NOT be diluted!

TEST PROCEDURE

STEP A:

Pipet 100 µL of 1:200 diluted samples, **PV** sample diluent (blank) and each **STA** standard solution (4 x 2 mL vials with green, yellow, blue and red lid) into the wells (all in duplicates).

INCUBATION: 45 minutes at 37°C.

STEP B:

Washing procedure: Empty the wells of the plate (dump or aspirate) and wash 3 x 300 µL per well with 1:10 diluted **WB** wash buffer. Pipet 100 µL of **CON** conjugate solution (blue lid) into all wells.

INCUBATION: 30 minutes at 37°C.

STEP C:

Danger!

Repeat washing procedure: Wash 3 x 300 µL per well with 1:10 diluted **WB** wash buffer. Pipet 100 µL of **TMB** substrate solution (black lid) into all wells.

INCUBATION: 20 minutes at 37°C (in the dark).

STEP D:

Danger!

Terminate the substrate reaction: Pipet rapidly 100 µL of the **STO** stop-solution (yellow lid) into all wells.

Measure the absorption at 450 nm (optional reference wavelength: 620 nm). The colour is stable for at least 60 minutes.

CUT-OFF levels suggested for TUB IgA ELISA:

- ⇨ **Less than 200 serounits:** **NEGATIVE**
- ⇨ **More than 300 serounits:** **POSITIVE**
- ⇨ **In-between 200 - 300 serounits:** **BORDERLINE**

	Please consult instructions for use				Consumables: use by … (expiry date)		Protect from moisture
	Manufacturer		Do not reuse		Do not use if damaged		
2°C 30°C	Store at 2 - 30°C	LOT	Batch code	Σ	Contains sufficient for <n> tests	REF	Catalogue number