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IVD | See external label | 2°C–8°C | Σ 96 tests | REF 5111-8

Mycobacterium Tuberculosis IgG (TB IgG)

REF **5111-8**

Enzyme immunoassay based on microtiter plate for the detection and quantitative determination of human IgG antibodies against **Mycobacterium tuberculosis** in serum and plasma

Test	**Mycobacterium Tuberculosis IgG ELISA**
Method	**ELISA: Enzyme Linked Immunosorbent Assay**
Principle	**Indirect ELISA : Antigen Coated Plate**
Cut-off	**10 U/mL**
Sample	**100µL**
Specificity	**99%**
Sensitivity	**100%**
Total Time	**~110min**
Shelf Life	**12 Months from the manufacturing date**

** Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account.*

INTENDED USE

The Diagnostic Automation, Inc. Mycobacterium tuberculosis IgG antibody ELISA kit has been designed for the detection and the quantitative determination of specific IgG antibodies against Mycobacterium tuberculosis in serum and plasma. Further applications in other body fluids are possible and can be requested from the Technical Service of Diagnostic Automation.

This assay is intended for in-vitro diagnostic use only.

Laboratory results can never be the only base of a medical report. The patient history and further tests have additionally to be taken into account.

GENERAL INFORMATION

Mycobacterioses (tuberculosis, leprosy, atypical mycobacterioses, paratuberculosis, and perhaps Crohn's Disease) are the infectious diseases of men and animals with the largest diffusion on earth. The infectious agents of tuberculosis are acid-resistant rod-like formed bacteria of the family Mycobacteriaceae, genus Mycobacterium. The germ was detected by Robert Koch in 1882. Owing to the very high infectious power of pathogenic mycobacteria, early diagnosis is essential to prevent spreading of the disease. Convergence of various approaches are necessary to control the mycobacterioses, immune reactions and bacterial shedding being variable during the diseases. However, usual diagnostic procedures were up to now unsatisfactory and did not allow to distinguish among different mycobacterial species. The illness is normally transferred by droplets of saliva from infected persons. The target of the infection are mostly the lungs, but also other organs like the brain, intestinal tract, bones, lymph nodes and kidneys can be afflicted. Tuberculosis is not only found in developing countries with 8 million of new infections yearly, but also in industrialized civilizations, as an actual disease with some thousands of cases yearly. Without treatment, the disease leads in 50% of the cases to death within less than two years. Clinical symptoms are fatigue, loss of weight, lack of appetite, light fever, nocturnal sweat and pain in the chest. Especially patients with HIV are threatened by tuberculosis due to their impaired immune system. A vaccination with living attenuated bacteria is possible (BCG = Bacille Calmette Guérin). This is mostly done with newborn or young children. With older patients, before the vaccination there is normally performed the tuberculin test (Pirquet or Mantoux), where a small amount of tuberculin is injected under the skin. In a positive case, there exist antibodies against Mycobacteria, and a vaccination is not necessary. Up to recently, there have not existed any serological methods to detect tuberculosis antibodies in serum. The only available procedure was besides the skin tuberculin test the direct microscopical identification of the dyed bacteria in sputum. Meanwhile specific antigens have been prepared either by purification of natural material or by recombinant methods. This ELISA test kit for the determination of IgG antibodies uses a cocktail of highly pure proteins in order to determine an immune response against the bacteria in human serum. A fresh or chronically active infection can be diagnosed by IgA and IgM tests, which are also available.

PRINCIPLE OF THE TEST

The Diagnostic Automation, Inc. Mycobacterium tuberculosis IgG antibody test kit is based on the principle of the enzyme immunoassay (EIA). Mycobacterium tuberculosis antigen is bound on the surface of the microtiter strips. Diluted patient serum or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized Mycobacterium tuberculosis antigen takes place. After a one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgG peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 20 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of IgG antibodies is directly proportional to the intensity of the color.

LIMITATIONS, PRECAUTIONS AND GENERAL COMMENTS

- Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed.
- All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken.
- Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly.
- All reagents have to be brought to room temperature (18 to 25 °C) before performing the test.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions.
- When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time.
- In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used.
- All reagents have to be used within the expiry period.
- In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation.
- The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

REAGENTS PROVIDED

Storage and Stability (refer to the expiry date on the outer box label)

Store kit components at 2-8°C. After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8°C. The opened kit should be used within three months.

Components	Volume / Qty.
Mycobacterium tuberculosis antigen coated microtiter strips	12
Calibrator A (Negative Control)	2 mL
Calibrator B (Cut-Off Standard)	2 mL
Calibrator C (Weak Positive Control)	2 mL
Calibrator D (Positive Control)	2 mL
Enzyme Conjugate	15 mL
Substrate	15 mL
Stop Solution	15 mL
Sample Diluent	60 mL
Washing Buffer (10×)	60 mL
Plastic foils	2
Plastic bag	1

1. Microtiter Strips
12 strips with 8 breakable wells each, coated with a M. tuberculosis antigen mixture (recombinant Mycobacterium tuberculosis antigens, with 18, 36 and 40 kDa). Ready-to-use.

2. Calibrator A (Negative Control)
2 mL, protein solution diluted with PBS, contains no IgG antibodies against Mycobacterium tuberculosis. Addition of 0.01 % methylisothiazolone and 0.01% bromonitrodioxane. Ready-to-use.

3. Calibrator B (Cut-Off Standard)
2 mL human serum diluted with PBS, contains a low concentration of IgG antibodies against Mycobacterium tuberculosis. Addition of 0.01% ethylisothiazolone and 0.01% bromonitrodioxane. Ready-to-use.

4. Calibrator C (Weak Positive Control)
2 mL, human serum diluted with PBS, contains a medium concentration of IgG antibodies against Mycobacterium tuberculosis. Addition of 0.01% ethylisothiazolone and 0.01% bromonitrodioxane. Ready-to-use.

5. Calibrator D (Positive Control)
2 mL, human serum diluted with PBS, contains a high concentration of IgG antibodies against Mycobacterium tuberculosis. Addition of 0.01% ethylisothiazolone and 0.01% bromonitrodioxane. Ready-to-use.

6. Enzyme Conjugate
15 mL, anti-human-IgG-HRP (rabbit), in protein-containing buffer solution. Addition of 0.01% methylisothiazolone and 0.01% bromonitrodioxane and 5 mg/L Proclin. Ready-to-use.

7. Substrate
15 mL, TMB (tetramethylbenzidine). Ready-to-use.

8. Stop Solution
15 mL, 0.5 M sulfuric acid. Ready-to-use.

9. Sample Diluent
60 mL, PBS/BSA buffer. Addition of 0.095 % sodium azide. Ready-to-use.

10. Washing Buffer
60 mL, PBS + Tween 20, 10x concentrate. Final concentration: dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

11. Plastic Foils
2 pieces to cover the microtiter strips during the incubation.

12. Plastic Bag
Resealable, for the dry storage of non-used strips.

MATERIALS REQUIRED BUT NOT PROVIDED

- 5 µL-, 100 µL- and 500 µL micro- and multichannel pipets
- Microtiter Plate Reader (450 nm)
- Microtiter Plate Washer
- Reagent tubes for the serum dilution
- Deionized water

SPECIMEN COLLECTION AND HANDLING

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 7 days.

For a longer storage they should be kept at -20°C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.

For the performance of the test the samples (not the standards) have to be diluted 1:101 with ready-to-use sample diluent (e.g. 5 µL serum + 500 µL sample diluent).

ASSAY PROCEDURE

1. Preparation of Reagents

Washing Solution: dilute before use 1+9 with deionized water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

- Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.
- All reagents and samples must be brought to room temperature before use, but should not be left at this temperature longer than necessary.
- A standard curve should be established with each assay.
- Return the unused microtiter strips to the plastic bag and store them dry at 2-8°C.

2. Assay Steps

2.1 Prepare a sufficient amount of microtiter wells for the standards, controls and samples as well as for a substrate blank.

2.2 Pipet 100 µL each of the **diluted** (1:101) samples and the **ready-to-use** standards and controls respectively into the wells. Leave one well empty for the substrate blank.

2.3 Cover plate with the enclosed foil and incubate at room temperature for 60 minutes.

2.4 Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.

2.5 Pipet 100 µL each of ready-to-use conjugate into the wells. Leave one well empty for the substrate blank.

2.6 Cover plate with the enclosed foil and incubate at room temperature for 30 minutes.

2.7 Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.

2.8 Pipet 100 µL each of the ready-to-use substrate into the wells. This time also the substrate blank is pipetted.

2.9 Cover plate with the enclosed foil and incubate at room temperature for 20 minutes in the dark (e.g. drawer).

2.10 To terminate the substrate reaction, pipet 100 µL each of the ready-to-use stop solution into the wells. Pipet also the substrate blank.

2.11 After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm (optionally reference wavelength of 620 nm). The color is stable for at least 60 minutes.

EVALUATION

Example

	OD Value	corrected OD
Substrate Blank	0.020	

Negative Control	0.024	0.004
Cut-Off Standard	0.520	0.500
Weak Positive Control	1.100	1.080
Positive Control	1.500	1.480

The above table contains only an example, which was achieved under arbitrary temperature and environmental conditions. The described data constitute consequently **no reference values** which have to be found in other laboratories in the same way.

1. Qualitative Evaluation

The calculated absorptions for the patient sera, as mentioned above, are compared with the value for the cut-off standard. If the value of the sample is higher, there is a positive result.For a value below the cut-off standard, there is a negative result. It seems reasonable to define a range of +/- 20 % around the value of the cut-off as a grey zone. In such a case the repetition of the test with the same serum or with a new sample of the same patient, taken after 2-4 weeks, is recommended. Both samples should be measured in parallel in the same run.

The positive control must show at least the double absorption compared with the cut-off standard.

2. Quantitative Evaluation

The ready-to-use standards and controls of the Mycobacterium antibody kit are defined and expressed in arbitrary units (U/mL). This results in an exact and reproducible quantitative evaluation. Consequently for a given patient follow-up controls become possible. The values for controls and standards in units are printed on the labels of the vials.

For a quantitative evaluation the absorptions of the standards and controls are graphically drawn against their concentrations. From the resulting reference curve the concentration values for each patient sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs. As curve fit point-to-point has to be chosen.
Calibrator B with its concentrations of 10U/mL serves as cut-off standard. Analogous to the qualitative evaluation a range of +/20% around the cut-off is defined as grey zone. Thus results between 8 and 12 U/mL are reported as borderline.

3. Interpretation

Although antibody may occur in healthy persons in rare cases, normally they indicate the colonisation with Mycobacterium tuberculosis. Positive IgM results are related to the early stage of an infection. During its course a seroconversion towards IgG antibodies take place. The simultaneous presence of IgG and IgM antibodies denote an infection at its early stage or a reactivation in chronic infections. The sole occurrence of IgG is a sign for a completed immunological response. IgA antibodies occur after the initial activation of immunoreaction as indicated by the presence of IgM and are associated to a high inflammatory potential. Since they are not affected by energy effects as Igg antibodies, they are useful marker for patients which show a reduced IgG response due to pre existing immune depression.

Briefly the humoral response can be summarized as follows:

- IgM (-) IgG (-) IgA (-) no infection
- IgM (+) IgG (-) IgA (-) infection at a very early stage
- IgM (-) IgG (+) IgA (+/-) completed infection
- IgM (+) IgG (+) IgA (+/-) infection at an early stage or re-infection
- IgM (-) IgG (-) IgA (+) completed infection in patients suffering from IgG reducing effects.

PERFORMANCE CHARACTERISTICS

Mycobacterium ELISA	IgG	IgA	IgM
Intra-Assay-Precision	7.6 %	7.9 %	7.9 %
Inter-Assay-Precision	9.4 %	7.4 %	7.4 %
Inter-Lot-Precision	3.1 – 9.9 %	5.7 – 8.9 %	5.7 – 8.9 %
Analytical Sensitivity	1.09 U/mL	1.34 U/ml	1.22 U/mL
Recovery	86 – 95 %	87 – 96 %	87 – 91 %
Linearity	82 – 113 %	78 – 111 %	78 – 118 %
Cross-Reactivity	No cross-reactivity to Helicobacter pylori and Bordetella pertussis.		
Interferences	No interferences to bilirubin up to 0.3 mg/mL, hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL		
Clinical Specificity	99 %	99 %	100 %
Clinical Sensitivity	100 %	100 %	100 %

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