Mycobacterium Tuberculosis ELISA IgG, IgM, IgA Assay (Serum/Plasma)

Instruction for use
Mycobacterium Tuberculosis
ELISA IgG, IgM, IgA Assay
(Serum/Plasma)

For Research Diagnostic Use
FOR RESEARCH OR INVESTIGATIONAL USE ONLY

INTENDED USE
The GenWay Biotech, Inc. *Mycobacterium tuberculosis* (TB) ELISA IgG, IgM, IgA Assay system is an enzyme-linked immunosorbent assay (ELISA) designed for the presumptive qualitative detection of IgG, IgM and IgA antibodies to *TB* in human serum and for the presumptive diagnosis of acute, recent, or reactive *TB* infection. To adequately assess the patient’s serological status, testing must be performed in conjunction with any of the other traditional TB diagnostic technologies.

SIGNIFICANCE AND BACKGROUND
The *Mycobacterium tuberculosis* belongs to the genus Mycobacterium, which are aerobic, non-motile, and rod-shaped bacteria with two distinguishing characteristics: acid-fastness and slow growth.

*Mycobacterium tuberculosis* is one of most successful pathogens of mankind, infecting one-third of the global population and claiming two million lives every year. The ability of the bacteria to persist in the form of a long-term asymptomatic infection, referred to as latent tuberculosis, is central to the biology of the disease.

Approximately eight million people develop active tuberculosis (TB) every year, with two million dying from the disease. In addition to this already huge burden of disease, it is estimated that up to two billion people have been infected with the causative agent, *Mycobacterium tuberculosis*. Most people control the initial infection by mounting a cell-mediated immune response that prevents disease but can leave a residual population of viable mycobacteria. Between 5 - 10% of individuals who become infected subsequently develop clinical disease. Primary TB develops within 1 or 2 years after an initial infection and, particularly in children, is often associated with disseminated disease. Post-primary TB develops later in life, and can be caused either by reactivation of bacteria remaining from the initial infection or by failure to control a subsequent reinfection. Post-primary TB is predominantly a pulmonary disease, involving extensive damage to the lungs and efficient aerosol transmission of bacteria. The risk of disease is highly dependent on the immune status of the host; coinfection with HIV markedly increases the incidence of both forms of disease.

Currently, latent infection is diagnosed in a non-immunized person by a tuberculin skin test, which yields a delayed hypersensitivity type response to an extract made from *M. tuberculosis*. Those immunized for TB or with past-cleared infection will respond with delayed hypersensitivity parallel to those currently in a state of infection, so the test must be used with caution, particularly with regard to persons from countries where TB immunization is common. Tuberculin tests have the disadvantage in that they may produce false negatives, especially when the patient is co-morbid...
with sarcoidosis, Hodgkins lymphoma, malnutrition, or most notably active tuberculosis disease. New TB tests are being developed that offer the hope of a less expensive, fast and more accurate TB testing. These use polymerase chain reaction detection of bacterial DNA and antibody assays to detect the release of interferon gamma in response to mycobacteria. These tests are not affected by immunization, so generate fewer false positive results.

Unlike the Tuberculin skin test, the GenWay Biotech, Inc. Mycobacterium Tuberculosis ELISA IgG, IgM, IgA Assay (Serum/Plasma) will detect HIV/TB coinfection and has no detectable cross reactivity with BCG vaccinations.

**PRINCIPLE OF THE ELISA ASSAY**

The GenWay Bio ELISA test system is designed to detect IgG, IgM, and IgA class antibodies to TB in human sera. Wells of plastic microwell strips are sensitized by passive absorption with TB composite antigen. The antigen composite is a combination of recombinant proteins and fractionated Mycobacterium components. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.

2. Peroxidase Conjugated Goat anti-Human IgG (γ-chain specific), Peroxidase Conjugated Goat anti-Human IgM (μ-chain specific) or Peroxidase Conjugated Goat anti-Human IgA (α-chain specific) is added to the wells and the plate is incubated. The Conjugate will react with IgG, IgM or IgA antibody immobilized on the solid phase in step 1. The wells are washed to remove unbounded Conjugate.

3. The microwells containing immobilized peroxidase Conjugate are incubated with TMB Peroxidase Substrate. Hydrolysis of the Substrate by peroxidase produces a color change. After a period of time the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the original test sample.

**MATERIALS PROVIDED**

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on packaging label.

<table>
<thead>
<tr>
<th><strong>PLATE</strong></th>
<th>96 wells configured in twelve 1 x 8 well strips coated with TB Composite. The strips are packed and sealed in a pouch with desiccant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CONJUGATE (15.0mL)</strong></td>
<td>One bottle containing ready-to-use Goat anti-Human IgA, IgG or IgA conjugated to Peroxidase.</td>
</tr>
<tr>
<td><strong>SPECIMEN DILUENT (30.0mL)</strong></td>
<td>One bottle containing ready-to-use IgA, IgG or IgM Specimen Diluent.</td>
</tr>
<tr>
<td><strong>TMB PEROXIDASE SUBSTRATE (15.0mL)</strong></td>
<td>One bottle containing ready-to-use TMB Peroxidase Substrate.</td>
</tr>
</tbody>
</table>
STOP SOLUTION (10.0mL) | One bottle containing ready-to-use Stop Solution.
---|---
WASH BUFFER (25mL) | One bottle of 20X Wash Buffer. Dilute the 20X Wash Buffer in 475mL of Reagent Grade Water.
NEGATIVE CONTROL (0.2mL) | Human serum. One vial with a green cap, ready-to-use.
POSITIVE CONTROL (0.2) | Human serum. One vial with a red cap, ready-to-use.

**NOTE:** The 20x Wash Buffer should be removed from the kit and stored at room temperature:

Once the 1X Wash Buffer has been prepared according to the directions, it should be stored at 2-8°C.

**Important Note:** All kit components and serum samples should be allowed to equilibrate to room temperature before use.

**Note: Kit also contains:**
1. Component list containing lot specific information is inside the kit box.
2. Package insert providing instructions for use.

**PRECAUTIONS**
1. For research use.
2. Normal precautions exercised in handling laboratory reagents should be followed. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA plate do not contain viable organisms. However, the strips should be considered POTENTIALLY BIOHAZARDOUS MATERIALS and handled accordingly.
4. The human serum controls are POTENTIALLY BIOHAZARDOUS MATERIALS. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual “Biosafety in Microbiological and Biomedical Laboratories”: current edition; and OSHA’s Standard for Blood borne Pathogens.
5. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach a room temperature before starting the assay.** Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
7. The Stop Solution is TOXIC. Causes burns. Toxic by inhalation, in contact with skin and if swallowed. In case of an accident or if you feel unwell, seek medical advice immediately.
8. The TMB Peroxidase Substrate is HARMFUL. Irritating to eyes, respiratory system and skin.
9. The Wash Buffer concentrate is an IRRITANT. Irritating to eyes, respiratory system and skin.
10. Wipe bottom of plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
11. Reagents from other sources or manufacturers should not be used.
12. TMB Peroxidase Substrate Solution should be colorless, very pale yellow, very pale green or very pale blue when used. Contamination of the TMB with conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color. To help reduce the possibility of contamination, refer to Test Procedure, Substrate Incubation section to determine the amount of TMB to be used and dispense into a secondary container only what is needed to properly perform the assay.
13. Dilution or adulteration of these reagents may generate erroneous results.
14. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
15. Avoid microbial contamination of reagents. Incorrect results may occur.
16. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
17. Avoid splashing or generating aerosols.
18. Do not expose reagents to strong light during storage or incubation.
19. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
20. Wash solution should be collected in a disposal basin. Treat the waste solution with 10% household bleach (0.5% sodium hypochlorite). Avoid exposure of reagents to bleach fumes.
21. Caution: Liquid waste at acid pH should be neutralized before adding to bleach solution.
22. Do not allow the conjugates to come in contact with containers or instruments that may have previously contained a solution utilizing sodium azide as a preservative. Residual amounts of sodium azide may destroy the conjugate’s enzymatic activity.
23. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this kit.

MATERIALS REQUIRED BUT NOT PROVIDED:
- ELISA microwell reader capable of reading at a wavelength of 450nm with a reference of 630nm.
- Pipettes capable of accurately delivering 10µL to 200µL.
- Multichannel pipette capable of accurately delivering (50µL -300µL)
- Reagent reservoirs for multichannel pipettes.
- Wash bottle or microwell washing system.
- Reagent grade water.
- One liter graduated cylinder.
- Serological pipettes.
- Disposable pipette tips.
- Laboratory timer to monitor incubation steps.
- Disposal basin and disinfectant. (Example:10% household bleach, 0.5% sodium hypochlorite.)
- Sample dilution plate

STORAGE CONDITIONS
1. Store the unopened kit between 2-8°C (with the exception of the 20X Wash Buffer concentrate which should be removed from the kit and stored at room temperature).
2. Coated microwell strips: Store between 2-8°C. Extra strips should be immediately resealed with desiccant and returned to proper storage.
3. Conjugate: Store between 2-8°C. DO NOT FREEZE.
4. Positive Control and Negative Control: Store between 2-8°C.
5. TMB Peroxidase Substrate: Store between 2-8°C.
6. Wash Buffer concentrate (20X): Store between 15-25°C. Diluted wash buffer (1X) should be stored between 2-8°C.
7. Specimen Diluent. Store between 2-8°C.
8. Stop Solution: Store between 2-8°C.

SPECIMEN COLLECTION
1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29-A3: Protection of Laboratory Workers from Infectious Disease.

2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
3. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay. Avoid using hemolysed, lipemic, or bacterially contaminated sera.
4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2°C and 8°C for no longer than 48 hours. If delay in testing is anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles that may cause loss of antibody activity and give erroneous results.

GENERAL PROCEDURE
1. Remove the individual components from storage and allow them to equilibrate to room temperature.
2. Determine the number of microwells needed. Allow for three Control determinations, one Blank, one Negative Control and one Positive Control, per run. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2-8°C.

<table>
<thead>
<tr>
<th>STRIP1</th>
<th>STRIP 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Patient 6</td>
</tr>
<tr>
<td>B</td>
<td>Patient 7</td>
</tr>
<tr>
<td>C</td>
<td>Pos. Control</td>
</tr>
<tr>
<td>D</td>
<td>Patient 1</td>
</tr>
<tr>
<td>E</td>
<td>Patient 2</td>
</tr>
<tr>
<td>F</td>
<td>Patient 3</td>
</tr>
<tr>
<td>G</td>
<td>Patient 4</td>
</tr>
<tr>
<td>H</td>
<td>Patient 5</td>
</tr>
</tbody>
</table>

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of Specimen Diluent) of the Negative Control, Positive Control, and each patient serum.
4. Add 100µL of Specimen Diluent to well A1 as a reagent blank. Check reader template requirements for the correct reagent blank well configuration.
5. To individual wells, add 100µL of each diluted control, and sample. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
6. Incubate the plate between 20-23°C using an incubator for 60 ± 5 minutes.
7. Wash the microwell strips 5 times with 1X Wash Buffer.
   Preparation of 1X wash buffer from 20X Wash Buffer concentrate – add 25mL of 20X Wash Buffer concentrate to 475mL reagent grade water.

A. Manual Wash Procedure:
   a. Vigorously shake out the liquid from the wells.
   b. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
   c. Repeat steps a. and b. for a total of 5 washes.
   d. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with 0.5% sodium hypochlorite (bleach) at the end of the days run.

B. Automated Wash Procedure:
   If using an automated microwell wash system, set the dispensing volume to 300 µL/well. Set the wash cycle for 5 washes with no delay between washes. After the washes invert the plate over a paper towel and tap firmly to remove any residual wash solution from the microwells.

8. Add 100µL of the Conjugate, either IgG, IgM, or IgA to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
9. Incubate the plate between 20-23°C using an incubator for 60 ± 5 minutes
10. Wash the microwells by following the procedure as described in step 7.
11. Add 100µL of TMB Peroxidase Substrate to each well, including reagent blank well, at the same rate and in the same order as the specimens were added. The substrate is a light sensitive substance. It should not be allowed to incubate under a direct or indirect light source and should be covered directly after application.

12. Incubate the plate between 20-23°C using an incubator for 14 to 16 minutes in the dark.
13. Stop the reaction by adding 50µL of Stop Solution to each well, including reagent blank well, at the same rate and in the same order as the TMB Peroxidase Substrate was added.
   Wells will turn from blue to yellow depending on their reactivities. After adding the Stop Solution, lightly tap the plate several times to ensure that the samples are thoroughly mixed.
14. Set the microwell reader to read at a wavelength of 450nm referencing 630 nm and measure the optical density (OD) of each well against the reagent blank. The plate should be read within 30 minutes after the addition of the Stop Solution.

ABBREVIATED TEST PROCEDURE
1. Dilute Serum 1:21
2. Add diluted serum to microwell 100 µL/well
3. Incubate for 60 to 65 minutes between 20-23°C using an incubator.
4. Wash
5. Add Conjugate – 100 µL/well
6. Incubate for 60 to 65 minutes between 20-23°C using an incubator
7. Wash
8. Add TMB 100 µL/well
9. Incubate for 14 to 16 minutes between 20-23°C using an incubator in the dark.
10. Add Stop Solution 50 µL/well - Mix
11. READ at 450nm reference 630nm

QUALITY CONTROL

1. Each time the assay is run a reagent blank, Negative Control, and Positive Control must also be included in each assay.
2. The OD value for the Positive and Negative Controls should fall within the following ranges:

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Positive Control (OD)</th>
<th>Negative Control (OD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>&gt; 0.300</td>
<td>&lt; 0.250</td>
</tr>
<tr>
<td>IgM</td>
<td>&gt; 0.150</td>
<td>&lt; 0.100</td>
</tr>
<tr>
<td>IgA</td>
<td>&gt; 0.250</td>
<td>&lt; 0.200</td>
</tr>
</tbody>
</table>

3. Run validity is determined through the performance of the positive and negative controls as well as the blank.
4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure and will not ensure precision at the assay cut-off.
5. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

Interpretations:

Optical Density (OD) ratios are interpreted as follows:

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Negative</th>
<th>Equivocal</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>&lt; 0.250</td>
<td>0.250 – 0.299</td>
<td>≥ 0.300</td>
</tr>
<tr>
<td>IgM</td>
<td>&lt; 0.100</td>
<td>0.100 – 0.149</td>
<td>≥ 0.150</td>
</tr>
<tr>
<td>IgA</td>
<td>&lt;0.200</td>
<td>0.200 – 0.249</td>
<td>≥ 0.250</td>
</tr>
</tbody>
</table>

1. An OD ratio < 0.250 indicates no detectable IgG antibodies to TB. An OD ratio ≥ 0.300 is reactive for IgG antibodies to TB. Specimens with OD ratio values in the equivocal range (0.250 – 0.299) should be retested. Specimens that remain in the equivocal range after repeat testing should be tested by an alternate serologic procedure.
2. An OD ratio < 0.100 indicates no detectable IgM antibodies to TB. An OD ratio > 0.150 is reactive for IgM antibodies to TB. Specimens with OD ratio values in the equivocal range (0.100 – 0.149) should be retested. Specimens that remain in the equivocal range after repeat testing should be tested by an alternate serologic procedure.

3. An OD ratio < 0.200 indicates no detectable IgA antibodies to TB. An OD ratio > 0.250 is reactive for IgA antibodies to TB. Specimens with OD ratio values in the equivocal range (0.200 – 0.249) should be retested. Specimens that remain in the equivocal range after repeat testing should be tested by an alternate serologic procedure.

LIMITATION OF THE ASSAY
1. A diagnosis should not be made on the basis of anti-TB results alone. Test results for anti-TB should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.
2. The use of hemolytic, lipemic, bacterially contaminated or heat inactivated specimens should be avoided. Erroneous results may occur.
3. A diagnosis should not be made on the basis of anti-EA results alone.
4. The assay performance characteristics have not been established for matrices other than sera.
5. The magnitude of the measured result above the cut-off is not indicative of the total amount of antibody present.
6. Assay performance characteristics have not been established for visual result determinations.
7. Caution should be used when evaluating samples obtained from immunosuppressed patients.

PERFORMANCE CHARACTERISTICS
Clinical evaluation was conducted comparing the results obtained using the GenWay Biotech, Inc. Mycobacterium Tuberculosis ELISA IgG, IgM, IgA Assay to a predicate device. The study included 163 serum samples from Russia, Ukraine, Togo, United States, and Northern Europe. 77 samples were identified as negative and 86 samples were identified as positive for pulmonary tuberculosis. Out of the 77 negative samples, 57 samples were retrieved from patients who had previously been BCG inoculated.

<table>
<thead>
<tr>
<th>PREDICATE DEVICE</th>
<th>GenWay Biotech, Inc. ELISA Method</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>77</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
</tbody>
</table>

The results yielded a Specificity of 100% and a Sensitivity of 89.5%. The test does not show positive results with BCG inoculated persons, and TB negative sera. The test also shows negative results with M. bovis infection.
REFERENCES


5. CLSI/NCCLS M29-A3: Protection of Laboratory Workers from Infectious Disease. Third Edition. Published by the Clinical Laboratory Standards Institute 3/11/05
