The Syphilis Total Antibody EIA Test Kit is a one step enzyme immunoassay for the qualitative detection of total antibodies (IgG, IgM and IgA) to Treponema Pallidum (TP) in human serum or plasma.

**INTENDED USE**

The Syphilis Total Antibody EIA Test Kit is a one step enzyme immunoassay for the qualitative detection of total antibodies (IgG, IgM and IgA) to Treponema Pallidum (TP) in human serum or plasma. It is intended for screening and as a confirmatory diagnosis of possible Syphilis infection.

**SUMMARY**

Treponema Pallidum (TP) is the causative agent of the venereal disease, Syphilis. TP is a spirochete bacterium with an outer envelope and cytoplasmic membrane. Relative little is known about the bacterium can be detected within 4 to 7 days after the chancre appears. The absence of uric acid from the product. If the specimen antibodies present to T. Pallidum, the complexes will not be formed. After initial incubation, the microwell plate is washed to remove unbound and substrate B are added and developed to produce a blue color indicating the amount of T. Pallidum antibodies present in the specimen. Sulfuric acid solution is added to the microwell plate to stop the reaction producing a color change from blue to yellow. The color intensity which corresponds to the amount of T. Pallidum antibodies present in the specimen is measured with a microplate reader at 450/630-700 nm or 450 nm.

**PRINCIPLE**

The Syphilis Total Antibody EIA Test Kit is a solid phase qualitative enzyme immunoassay based on a sandwich principle for the detection of total antibodies (IgG, IgM and IgA) to Treponema Pallidum in human serum or plasma. The microwell plate is coated with recombinant antigens for T. Pallidum. During testing, the specimen and the enzyme-conjugated antigen coated microwell plate will be exposed to the microwell plate. The antigen coated microwell plate and substrate B are added and developed to produce a blue color indicating the amount of T. Pallidum antibodies present in the specimen. Sulfuric acid solution is added to the microwell plate to stop the reaction producing a color change from blue to yellow. The color intensity which corresponds to the amount of T. Pallidum antibodies present in the specimen is measured with a microplate reader at 450/630-700 nm or 450 nm.

The Syphilis Total Antibody EIA Test Kit can be used for the following assays:

- Detecting total antibodies (IgG, IgM and IgA) to Treponema Pallidum in human serum or plasma.
- Determining the presence of Treponema Pallidum in specimens.
- Evaluating the effectiveness of treatment for Treponema Pallidum infection.

**SPECIMEN COLLECTION AND PREPARATION**

- The specimen must be collected from a venous blood sample using a sterile syringe and needle.
- The specimen should be collected in a sterile container and placed on ice immediately after collection.
- The specimen should be transported to the laboratory within 4 hours of collection.
- The specimen should be stored at 2-8°C upon receipt.

**REAGENT COMPONENTS**

- **Microwell Plate**: Microwell plate coated with recombinant antigens for T. Pallidum.
- **Conjugate**: Enzyme-conjugated antigen.
- **Concentrated Wash Buffer**: 1M sodium chloride.
- **Substrate A**: Tris-HCl buffer.
- **Substrate B**: TMB.
- **Stop Solution**: 0.5M sulfuric acid.

**REAGENT DESCRIPTIONS AND MATERIALS PROVIDED**

<table>
<thead>
<tr>
<th>No.</th>
<th>Reagent</th>
<th>Component Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Syphilis Control Plate</td>
<td>Microwell plate coated with T. Pallidum antigens</td>
<td>1 plate (96 wells/plate)</td>
</tr>
<tr>
<td>2</td>
<td>Syphilis Conjugate</td>
<td>Recombinant T. Pallidum antigens bound to peroxidase</td>
<td>1 x 1 mL</td>
</tr>
<tr>
<td>3</td>
<td>Concentrated Wash Buffer (25x)</td>
<td>Tris-HCl buffer</td>
<td>1 x 40 mL</td>
</tr>
<tr>
<td>4</td>
<td>Substrate A</td>
<td>Citrate-phosphate buffer containing TMB and H2O2</td>
<td>1 x 5 mL</td>
</tr>
<tr>
<td>5</td>
<td>Substrate B</td>
<td>Containing tetrathylbenzene (TMB)</td>
<td>1 x 5 mL</td>
</tr>
<tr>
<td>6</td>
<td>Syphilis Negative Control</td>
<td>Normal serum non-reactive for syphilis, HIV, HBAg, HCV, and hepatitis B</td>
<td>1 x 1 mL, 5 x 1 mL</td>
</tr>
<tr>
<td>7</td>
<td>Syphilis Positive Control</td>
<td>Reactivated serum containing antibodies to Treponema Pallidum (TP)</td>
<td>1 x 1 mL, 5 x 1 mL</td>
</tr>
</tbody>
</table>

**DIRECTIONS FOR USE**

1. Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25. The Working Wash Buffer is stable for 2 weeks at 2-8°C.
2. Add 50 μL of Positive Control in wells B1 and C1. (Red Reagent).
3. Add 50 μL of Test strips to wells B1 and C1. (Red Reagent).
4. Add 50 μL of Positive Control in wells B1 and C1. (Red Reagent).
5. Add 50 μL of Test strips to wells B1 and C1. (Red Reagent).

**REAGENTS AND MATERIALS PROVIDED**

<table>
<thead>
<tr>
<th>No.</th>
<th>Reagent</th>
<th>Component Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Syphilis Control Plate</td>
<td>Microwell plate coated with T. Pallidum antigens</td>
<td>1 plate (96 wells/plate)</td>
</tr>
<tr>
<td>2</td>
<td>Syphilis Conjugate</td>
<td>Recombinant T. Pallidum antigens bound to peroxidase</td>
<td>1 x 1 mL</td>
</tr>
<tr>
<td>3</td>
<td>Concentrated Wash Buffer (25x)</td>
<td>Tris-HCl buffer</td>
<td>1 x 40 mL</td>
</tr>
<tr>
<td>4</td>
<td>Substrate A</td>
<td>Citrate-phosphate buffer containing TMB and H2O2</td>
<td>1 x 5 mL</td>
</tr>
<tr>
<td>5</td>
<td>Substrate B</td>
<td>Containing tetrathylbenzene (TMB)</td>
<td>1 x 5 mL</td>
</tr>
<tr>
<td>6</td>
<td>Stop Solution</td>
<td>0.5M sulfuric acid</td>
<td>1 x 5 mL</td>
</tr>
</tbody>
</table>

**MATERIALS REQUIRED BUT NOT PROVIDED**

- 10 μL of Negative Control in wells B1 and C1.
- 10 μL of Positive Control in wells B1 and C1.
- 50 μL of Test strips to wells B1 and C1.
- 50 μL of Test strips to wells B1 and C1.
- 50 μL of Test strips to wells B1 and C1.

**PROTOCOL**

1. Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
2. Do not expose reagents especially the Substrate to strong light or hypochlorite fumes during storage or incubation steps.
3. Do not store Stop Solution in a shallow dish or return it to the original bottle after use.
4. Wash and dry the plate before adding reagents.
5. Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25. The Working Wash Buffer is stable for 2 weeks at 2-8°C.

**DIRECTIONS FOR USE**

1. Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25. The Working Wash Buffer is stable for 2 weeks at 2-8°C.
2. Add 50 μL of Positive Control in wells B1 and C1. (Red Reagent).
3. Add 50 μL of Test strips to wells B1 and C1. (Red Reagent).
4. Add 50 μL of Positive Control in wells B1 and C1. (Red Reagent).
5. Add 50 μL of Test strips to wells B1 and C1. (Red Reagent).

**PRECAUTIONS**

- Do not touch the bottom of the wells with pipette tips. Make sure the bottom of the microwell plate with fingertips.
- Do not allow sodium hypochlorite fumes from chlorine bleach or other sources to contact the microwell plate during the assay as the reaction color may be inhibited.
- All equipment should be used with care, calibrated regularly and maintained following the equipment manufacturer’s instructions.

**HEALTH AND SAFETY INFORMATION**

- Some components of this kit contain human blood derivatives. No known test method can offer complete assurance that all body fluids and tissues are free from blood-borne infectious agents.
- Avoid the use of the kit or the disposal of its discarded materials by those who are allergic to human-derived materials.
- Wear protective clothing and eyewear when handling the reagents.

**STORAGE AND STABILITY**

- Store the reagents and specimen to reach room temperature (15-30°C) prior to testing. The procedure must be strictly followed. Assay must proceed to completion within time limits. Arrange for destruction of all unused reagents.
- The test procedure must be strictly followed. Assay must proceed to completion within time limits. Arrange for destruction of all unused reagents.
- Keep the reagents and specimen to reach room temperature (15-30°C) prior to testing. The procedure must be strictly followed. Assay must proceed to completion within time limits. Arrange for destruction of all unused reagents.
- Do not expose reagents especially the Substrate to strong light or hypochlorite fumes during storage or incubation steps.
- Do not store Stop Solution in a shallow dish or return it to the original bottle after use.

**CONSUMER INFORMATION**

- Some components of this kit contain human blood derivatives. No known test method can offer complete assurance that all body fluids and tissues are free from blood-borne infectious agents.
- Avoid the use of the kit or the disposal of its discarded materials by those who are allergic to human-derived materials.
- Wear protective clothing and eyewear when handling the reagents.

**DIRECTIONS FOR USE**

1. Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25. The Working Wash Buffer is stable for 2 weeks at 2-8°C.
2. Add 50 μL of Positive Control in wells B1 and C1. (Red Reagent).
3. Add 50 μL of Test strips to wells B1 and C1. (Red Reagent).
4. Add 50 μL of Positive Control in wells B1 and C1. (Red Reagent).
5. Add 50 μL of Test strips to wells B1 and C1. (Red Reagent).

**PRECAUTIONS**

- Do not touch the bottom of the wells with pipette tips. Make sure the bottom of the microwell plate with fingertips.
- Do not allow sodium hypochlorite fumes from chlorine bleach or other sources to contact the microwell plate during the assay as the reaction color may be inhibited.
- All equipment should be used with care, calibrated regularly and maintained following the equipment manufacturer’s instructions.
Mix gently then cover microwell plate with Plate Sealer and incubate in a water bath or incubator at 37°C ± 2°C for 15 minutes ± 1 minute.

Mix then cover microwell plate with Plate Sealer and incubate at 37°C for 15 min.

Remove the Plate Sealer.

Remove the Plate Sealer.

Add 50 µL of Stop Solution to each well. (Clear Reagent)

Then a yellow color should develop in wells containing Positive specimens.

Read at 450/630-700 nm within 30 minutes. Note: Microwell plate can also be read at 450 nm, but it is strongly recommended to read it at 450/630-700 nm for better results.

Read at 450/630-700 nm within 30 min.

Automated EIA microplate processors may be used to perform the assay after validating the results to ensure they are equivalent to those obtained using the manual method for the same specimens.

Incorporation times may vary depending on the instruments used but do not prolong incubation times than the procedure listed above. When automatic EIA microplate processors are used, periodic validation is recommended to ensure proper results.

Automatic EIA microplate processors may be used to perform the assay after validating the results to ensure they are equivalent to those obtained using the manual method for the same specimens.

Incorporation times may vary depending on the instruments used but do not prolong incubation times than the procedure listed above. When automatic EIA microplate processors are used, periodic validation is recommended to ensure proper results.

1. Calculate the Mean Absorbance of Negative Control and Positive Control by referring to the table below.

<table>
<thead>
<tr>
<th>Item</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control: Well B1</td>
<td>0.028</td>
</tr>
<tr>
<td>Negative Control: Well C1</td>
<td>0.030</td>
</tr>
<tr>
<td>Total Absorbance of Negative Control</td>
<td>0.028 ± 0.058</td>
</tr>
<tr>
<td>Mean Absorbance of Negative Control</td>
<td>0.058/3 ± 0.029</td>
</tr>
<tr>
<td>Blank Absorbance: Well A</td>
<td>0.008</td>
</tr>
<tr>
<td>Mean Absorbance of Negative Control – Blank Absorbance</td>
<td>0.029 – 0.058/0.021</td>
</tr>
</tbody>
</table>

2. Check the validation requirements below to determine if the test results are valid.

<table>
<thead>
<tr>
<th>Item</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Absorbance/ Cut-Off</td>
<td></td>
</tr>
<tr>
<td>Standard Deviation</td>
<td></td>
</tr>
<tr>
<td>Coefficient of Variation (%)</td>
<td></td>
</tr>
<tr>
<td>Mean Absorbance/ Cut-Off</td>
<td></td>
</tr>
<tr>
<td>Standard Deviation</td>
<td></td>
</tr>
<tr>
<td>Coefficient of Variation (%)</td>
<td></td>
</tr>
</tbody>
</table>

3. Calculate the Cut-Off Value using the following formula if the test results are valid.

**Example of Cut-Off Value Calculation**

<table>
<thead>
<tr>
<th>Item</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cut-Off Value: NCx + 0.14</td>
<td>0.021 - 0.140 ± 0.161</td>
</tr>
</tbody>
</table>

**INTERPRETATION OF RESULTS**

**NON-REACTIVE:** Specimens with absorbance less than the Cut-Off Value are considered non-reactive for antibodies to *T. Pallidum* and may be considered negative.

**REACTIVE:** Specimens with absorbance greater than or equal to the Cut-Off Value are considered initially reactive for antibodies to *T. Pallidum*. The specimen should be retested in duplicate before final interpretation. Specimens that are reactive in at least one of the re-tests are presumed to be repeatedly reactive and should be confirmed using confirmatory testing. Specimens that are non-reactive on both retests should be considered non-reactive.

**NOTE:** Specimens with values within 10% of the Cut-Off Value should be retested in duplicature for final interpretation.

**LIMITATIONS**

1. The Syphilis Total Antibody EIA Test Kit is used for the detection of *T. Pallidum* antibodies in human serum or plasma. Diagnosis of an infectious disease should not be established based on a single test result. Further testing, including confirmatory testing, should be performed before a specimen is considered positive. A non-reactive test result does not exclude the possibility of exposure. Specimens containing precipitate may give inconsistent test results.

2. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.

3. As with other sensitive immunoassays, there is the possibility that non-repeatable reaction may occur due to inadequate washing. The results may be affected due to procedural or instrument error.

4. The Positive Control in the test kit is not to be used to quantify assay sensitivity. The Positive Control is used to verify that the test kit components are capable of detecting a reactive specimen provided the procedure is followed as defined in the kit and the storage conditions have been strictly adhered to.

**PERFORMANCE CHARACTERISTICS**

Sensitivity and Specificity

The Syphilis Total Antibody EIA Test Kit has correctly identified specimens of a seroconversion panel and has been compared with a leading commercial TPPA Syphilis test using clinical specimens. The results show that the clinical sensitivity of the Syphilis Total Antibody EIA Test Kit is > 99.9%, and the clinical specificity is 99.9%.

**Syphilis Total Antibody EIA vs. TPPA**

<table>
<thead>
<tr>
<th>Method</th>
<th>TPPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results</td>
<td>Positive Negative Total Results</td>
</tr>
<tr>
<td>Positive</td>
<td>389</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td>Total Results</td>
<td>389</td>
</tr>
</tbody>
</table>

Clinical Sensitivity: >99.9% (99.1-100.0%)*

Clinical Specificity: 99.9% (99.7-99.9%)*

Overall Agreement: 99.9% (99.7-99.9%)*

*95% Confidence Interval

**BIBLIOGRAPHY**

1. Claire FM. Complete Genome Sequence of Treponema Pallidum, the Syphilis Spirochete, Science 1998; 281: July 375-381.


