Enzyme Immunoassay

**Syphilis IgG/IgM**

Cat # 1465Z

For *in vitro* Research Use Only

<table>
<thead>
<tr>
<th>Test</th>
<th>Syphilis IgG / IgM ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>ELISA: Enzyme Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>Principle</td>
<td>Indirect- ELISA; Antigen Coated Plate</td>
</tr>
<tr>
<td>Detection Range</td>
<td>Qualitative: Positive &amp; Negative Control</td>
</tr>
<tr>
<td>Sample</td>
<td>5µL</td>
</tr>
<tr>
<td>Specificity</td>
<td>Not observed</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Not observed</td>
</tr>
<tr>
<td>Total Time</td>
<td>~ 45 min</td>
</tr>
<tr>
<td>Shelf Life</td>
<td>12-14 months</td>
</tr>
</tbody>
</table>

*Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account.*
NAME AND INTENDED USE
The Diagnostic Automation Inc. Syphilis SS qualitative and semi-quantitative is a solid phase enzyme-linked immunosorbent assay (ELISA). This test provides an easy method for detecting Treponema pallidum antibodies (IgG & IgM) in human serum or plasma. (For Professional Use Only)

SUMMARY AND EXPLANATION OF TEST
Infection with Treponema pallidum, the caustic organism of syphilis, produced in humans at least two types of antibodies: (A) non-Treponemal antibodies or reagin which react with lipid antigens, and (B) Treponema antibodies, which react with Treponema and its closely related strains. Cardiolipin based flocculation test such as the Rapid Plasma regain (RPR) card test and the Venereal Disease Research Laboratory (VDRL)2 are commonly used in screening for syphilis. However, the sensitivities and specificities of such tests are less than that of the TPHA (Treponema pallidum Hemagglutination Assay). The screening results are usually confirmed by fluorescent-Treponemal antibody absorption test (FTA/ABS). (DAI syphilis) is an enzyme immunoassay for T. pallidum antibodies. Its sensitivity and specificity are sufficiently high to be in blood bank screening (automation) for syphilis, in estimation of primary, secondary or early latent syphilis and may be used in monitoring the progress of syphilis treatment. Sera giving positive results may be tested for T. pallidum IgM class antibodies.

DAI (IA-808) as an aid to the identification of active syphilis. Alternatively, for the purpose of screening high risk groups DAI Syphilis can be used in parallel with DAI Syphilis IgM (IA-808) in order to access immediately the full serological status of the patient.

PRINCIPLE OF THE ASSAY
DAI Syphilis is a solid phase enzyme-linked immunosorbent system employing plastic wells coated with T. pallidum antigens. Incubation of serum samples in the coated wells results in the binding of anti-Treponema pallidum antibodies to the immobilized antigens. Subsequent addition of the TP enzyme conjugate (peroxidase), is in direct proportion to the amount of Treponema pallidum antibody present in the serum sample. Unbound enzyme conjugate is washed from the wells and a substrate and chromogen solution is added. The intensity of the color formed as a result of enzyme activity is a direct measure of the anti-Treponema pallidum antibody present in the serum samples and may be quantified by use of a photometric well reader at 450 nm wavelength. Samples and may be quantified by use of microreader at 450 nm wavelength.

WARNING AND PRECAUTION
1. DAI Syphilis qualitative is designed for in vitro use only.
2. The components in this kit are intended for use as an integral unit. The components from different lots should not be mixed and used.
3. References contain human serum should be treated as potentially infectious. The references were found negative for Hepatitis B, C and HIV antibodies. However, because no test methods offer complete assurance of the absence of the HIV I/II, Hepatitis B, Hepatitis C virus or other infectious agents, these materials should be handled at the Biosafety Level 2 as recommended for any potentially infectious serum or blood specimen in the Center for Disease Control/National Institutes of Health Manual” Biosafety in Microbiological and Biomedical Laboratories”, 1984. Never pipet by mouth. Avoid contact with skin.

MATERIALS PROVIDED
1. Microwell Strips (96 wells): Recombinant T. pallidum Antigens coated wells. 8 x 12 strips.
2. Enzyme Conjugate (11mL): Treponema Pallidum antigens conjugated to horseradish peroxidase.
3. Sample Diluent (11mL)
4. Reference Control Set (0.8 mL each vial): Negative, Positive Control.
5. Washing Buffer Concentrate (100X) (10 ml): Prepare working solution by adding purified water to 1 liter.
6. TMB Substrate Solution (11mL): Buffer solution containing peroxide and TMB
7. Stop Solution: 2 N HCl.
8. Well holder with wells.

MATERIALS REQUIRED BUT NOT PROVIDED
1. Micro-well reader with wavelength at 450 nm.
2. Pipetor with tips for measuring 50 and 100 uL.
3. Clean plastic washing bottle of 1000 mL capacity for use in washing micro-wells with working washing buffer during testing procedure.

REAGENT PREPARATION
Prepare the working washing buffer by adding the entire contents of the Wash Buffer Concentrate to 1000 ml distilled water in a clean plastic wash bottle. Mix gently to dissolve. Store at room temperature.

STORAGE AND STABILITY
1. Store the kits at 2-8°C and keep micro-wells in a dry bag with desiccants.
2. Unopened reagents are stable until expiration of the kit. Solution A and Solution B should be colorless; if the solution turns blue, it must be replaced. Do not expose these reagents to strong light during storage or usage.
PRECAUTIONS
This kit is designed for in vitro diagnostic use only. The components of this kit are carefully matched and intended for use as an integral unit. Components of different lots should not be used interchangeably. Although all human materials used in the manufacture of this kit have been found negative for Hepatitis B antigen and for antibodies to HIV and HCV by required test methods, no test can offer complete assurance that infectious agents are not present, and therefore all calibrators, controls and samples should be handled as potentially infectious agents.

SPECIMEN COLLECTION AND HANDLING
Collect blood by venipuncture and allow clotting. Separate the serum by centrifugation at room temperature. Do not heat and inactivate serum. If sera cannot be immediately assayed, they may be stored at –20°C for at least six months. Avoid repeated freezing and thawing of samples. Specimens obviously contaminated with bacteria should not be use. Specimens turbid with high lipid concentrations should be clarified prior to assay.

PREPARATION FOR ASSAY
1. Bring all reagents and samples to room temperature (20-25°C) and mix gently before beginning the test.
2. Have all reagents and samples ready before the start of the assay. Once the test has begun it must be performed without any interruptions to get the most reliable and consistent results.
3. Use new disposable tips for each specimen.

ASSAY PROCEDURE (30/15)
A. Qualitative:
1. Secure the desired number of coated wells in the holder. Mark data sheet with sample identification.
2. Dispense 25 uL of sample diluent into well #1 as a blank, the Negative Control into well #2 and #3, The Positive Control into well #4 and #5, and the Patient Samples into the remaining wells in duplicate.
3. Dispense 100 uL of TP Enzyme Conjugate into each well except blank well.
4. Incubate for 30 minutes at room temperature.
5. Wash Five times with the Washing buffer.
6. Dispense 100 uL of TMB solution.
7. Incubate for 15 minutes at room temperature.
8. Stop reaction by adding 50 uL of Stop Solution in each well.
9. Zero a micro reader on the blank and measures absorbance of each well at 450 nm.

B. Quantitative:
1. Dispense 50 uL of the sample diluent into well 2 through well 8 etc
2. Dispense 50 uL of patient serum or plasma onto well 1 and 50 uL onto well 2.
3. Mix well 2 thoroughly then transfer 50 uL of the mixture from well 2 to well 3. Mix thoroughly. Repeat this dilution procedure to well 8. Discard 50 ul from the last well.

4. Well 1 through 8 represent a dilution series as follows:

<table>
<thead>
<tr>
<th>Well</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution</td>
<td>Undil</td>
<td>1:1</td>
<td>1:2</td>
<td>1:4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Well</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution</td>
<td>1:8</td>
<td>1:16</td>
<td>1:32</td>
<td>1:64</td>
</tr>
</tbody>
</table>

5. Proceed with the test as described in the Assay Procedure from Step 3 to Step 9.

**PROCEDURAL NOTE**

1. Wash the microwells and remove water thoroughly to get the best results.
2. Pipet all reagents and samples into bottom of the well. Vortex mixing or shaking of wells after sample and reagent pipeting is not required.
3. The appropriate number of wells should be secured in a holder and all reagent and sample caps should be removed prior to the start of testing. This will permit pipetting at equally intervals without interruption. A maximum of 30 patients’ samples should be assayed at one time in order to minimize error due to timing differences between specimens.

**INTERPRETATION OF RESULTS**

Specimens yielding absorbance reading greater than 0.3 should be reported as positive for antibodies against Treponema pallidum. Absorbance less than 0.3 within 10% limit, a new sample after two weeks should be retested with the old sample. If O.D. is less than 0.3, the sample is considered negative.

**VALIDATION OF TEST**

1. Negative Control: mean absorbance value should be <0.2 units.
2. Positive Control mean absorbance value should be greater than O.D. 1.0
3. A test may be validated if the above criteria are met.

**LIMITATIONS OF THE PROCEDURE**

The results obtained by means of this kit should be used as an aid for diagnosis and should not be interrupted as diagnostic by itself. Should negative results be obtained and other clinical findings suggest infection by Treponema pallidum, a second serum should be obtained two weeks after the first and testing repeated. Initial testing may have occurred prior to significant antibody production in response to infection.
QUALITY CONTROL
Each laboratory should utilize internal controls several levels to monitor assay performance. The controls should be treated as unknown. Results obtained should be in agreement with the assigned values of the control. Controls can be obtained from commercially available source. Controls should not contain sodium azide as preservatives.

PERFORMANCE CHARACTERISTICS
The DAI ELISA is tested for the established reactivity pattern against reference control. A total of 750 specimens were tested by approved commercial RPR card test and TPHA. The following results were obtained:

<table>
<thead>
<tr>
<th>No. of Samples</th>
<th>DAI ELISA</th>
<th>RPR</th>
<th>TPHA</th>
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</thead>
<tbody>
<tr>
<td>Total # of Assay Negative Samples</td>
<td>694</td>
<td>694</td>
<td>694</td>
</tr>
<tr>
<td># of Assay with reactive results</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td># of Assay with non-reactive results</td>
<td>694</td>
<td>694</td>
<td>694</td>
</tr>
<tr>
<td>Total # of Assay Nonspecific samples</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td># of Assay with reactive results</td>
<td>1</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td># of Assay with Negative Results</td>
<td>7</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Total # of Assay RPR reactive samples</td>
<td>48</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td># of Assay with reactive results</td>
<td>48</td>
<td>47</td>
<td>48</td>
</tr>
<tr>
<td># of Assay with non-reactive results</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

SPECIFICITY
DAI Syphilis IgG & M is specific. No cross reactivities found with the following tests: Toxoplasma IgG, IgM; CMV IgG, IgM; Rubella IgG, IgM, HSV I,II IgG, IgM, HCV, HBsAg, Cysticercosis, Chlamydia Trachomatis, Rubeola.

REFERENCES
<table>
<thead>
<tr>
<th>Date Adopted</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009-07-04</td>
<td>DA-Syphilis IgG/IgM-2009</td>
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**ISO 13485-2003**

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