RESULTS

Calculate the mean absorbance for each control and unknown.

Qualitative results:

If the absorbance of the sample is higher than that of the Cut-Off, the sample is positive for the presence of specific IgM. Calculate the ratio between the average OD value of the sample and that of the Cut-Off. The sample is considered:

- Positive: if the ratio is > 1.1.
- Doubtful: if +/- 10% of the Cut-Off.
- Negative: if the ratio is < 0.9.

If the result is doubtful, repeat the test. If it remains doubtful, collect a new serum sample.

LIMITATIONS OF THE PROCEDURE

- A serum sample obtained during the late phase of infection, when only IgG antibodies are present, may be negative by this procedure.
- The test result should be used in conjunction with information available from the evaluation of other procedures.
- Avoid repeated freezing and thawing of reagents and specimens.
- Grossly hemolyzed, icteric or lipemic specimens should be avoided.
- Heat inactivated sera should be avoided.

QUALITY CONTROL

Subtract the value of the blank from all the other readings. The OD values of Cut off control must be at least 0.2. Positive control must have an OD at least 1.5 times that of Cut off control.

PERFORMANCE CHARACTERISTICS

1. Sensitivity and Specificity

Out of 80 sera were analyzed by this Syphilis IgM Elisa and a commercial Elisa (Test A) as reference method. Out of 80 samples, 3 were positive for the presence of IgM antibodies to Treponema pallidum by Aviva Elisa and commercial Elisa also showed 3 of them as positive. The results are summarized below:

<table>
<thead>
<tr>
<th>Test</th>
<th>Positive</th>
<th>Negative</th>
<th>FN (false negative)</th>
<th>FP (false positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVIVA</td>
<td>3</td>
<td>77</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Test A</td>
<td>3</td>
<td>77</td>
<td>0</td>
<td>0</td>
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</table>

2. Precision

2. Inter-assay Study

<table>
<thead>
<tr>
<th>No of Replicates 16</th>
<th>Serum 1</th>
<th>Serum 2</th>
<th>Serum 3</th>
<th>Mean (OD's)</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.73</td>
<td>1.81</td>
<td>0.023</td>
<td>0.811</td>
<td>0.058</td>
<td>7.22</td>
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3. Intra-assay Study

<table>
<thead>
<tr>
<th>No of Replicates 16</th>
<th>Serum 1</th>
<th>Serum 2</th>
<th>Serum 3</th>
<th>Mean (OD's)</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.007</td>
<td>0.963</td>
<td>0.022</td>
<td>0.299</td>
<td>0.012</td>
<td>2.7</td>
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</tbody>
</table>

REFERENCE


For Research Purposes Only
Trexonema Antigen Coated Microtitration Strips
One strip holder containing 12x8 (96) microtitration wells coated with Treponema pallidum antigen. Store at 2-8°C until expiration date. Remove the support and strips to be used from the foil package, and place the unused strips in the polyethylene bag with the silica gel, expel the air and seal by pressing the closure. Once opened, the product is stable for 4 weeks at 2-8°C.

Wash Concentrate
One bottle, 100 mL, containing a phosphate buffered saline, concentrated 10-fold containing 0.5% per weight by volume polythene bag with the silica gel, expel the air and seal by pressing the closure. Once opened, the product is stable for 5 days at room temperature and 2 weeks at 2-8°C.

Sample Diluent
One bottle, 100 mL, containing a protein solution with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

Treponema IgM Controls
Three vials, negative, cut off and positive, each 2 mL of VDPSOH. Store at 2-8°C until expiration date.

2nd Antibody Conjugate
One bottle, 12 mL, containing anti-human IgM monoclonal antibodies labeled with peroxidase, in a phosphate buffer solution with 0.02% Proclin. Store at 2-8°C until expiration date.

Sorbent M
One Bottle, 4 ml containing protein solution in a phosphate buffer solution with 0.02% proclin. Store at 2-8°C.

TMB Substrate
One bottle, 12 mL, containing tetramethylenbenzidine (TMB) and hydrogen peroxide stabilized in citrate buffer, pH 3.8. Store at 2-8°C until expiration date.

STOPPING SOLUTION
One bottle, 15 mL, containing 0.3 M H2SO4 in solution. Store at 2-8°C until expiration date.

STORAGE SOLUTION
One bottle, 15 mL, containing 0.3 M H2SO4 in solution. Store at 2-8°C until expiration date.

PRECAUTIONS
For in vitro use
The following universal Good Laboratory Practices should be observed:

- Do not eat, drink, smoke or apply cosmetics where immunodiagnostic material is being handled. Do not pipet by mouth.
- Wear lab coats and disposable gloves when handling immunodiagnostic material. Wash hands thoroughly afterwards. Cover working area with disposable absorbent paper. Wipe up spills immediately and decontaminate affected surfaces. Avoid generation of aerosols. Provide adequate ventilation. Handle and dispose all reagents and materials in compliance with applicable regulations.

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL
This kit may contain some reagents made with sample source material (e.g. serum or plasma) or used in conjunction with sample source materials. The material in this kit has been tested by CE marked methods and found to be non-reactive for HIV-1/2 Antibodies, HCV and HBsAg. No available test method can offer complete assurance of eliminating potential biohazardous risk. Handle all reagents and samples at a Biosafety Level 2, as recommended for any potentially infectious sample material in the Centers for Disease Control/National Institutes of Health manual "Biologicals in Microbiological and Biomedical Laboratories," 4th Edition, April 1999.

WARNING AND PRECAUTION
Some of the reagents in this kit contain sodium azide as a preservative at concentrations below the regulatory limit of <0.1%. Although significantly diluted, concentrated sodium azide is an irritant to skin and mucous membranes, and may react with lead and copper plumbing to form explosive metal azides, especially if accumulated. Additionally, TMB and Sulfuric Acid, in concentrated amounts are also irritants to skin and mucous membranes. These substances are in diluted form and therefore may minimize exposure risks significantly but not completely. Provide adequate ventilation. Avoid contact with skin or mucous membranes. Avoid contact with any of these reagents, wash thoroughly with water and seek medical advice. Dispose all nonhazardous reagents by flushing with large volumes of water to prevent buildup of chemical hazards in the plumbing system.

For further information regarding hazardous substances in the kit, please refer to the component specific MSDS by request.

SPECIMEN COLLECTION AND HANDLING
Serum should be used, and the usual precautions for venipuncture should be observed. Specimens may be stored at 2-8°C for 2 days. For longer periods, store at -20°C. Do not use hemolyzed or lipemic specimens. Avoid repeated freezing and thawing of samples.

PREPARATION FOR ASSAY
A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert. Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion. Do not mix various lots of any kit component within an individual assay. Do not apply any component beyond the expiration date shown on its label. Incomplete washing will adversely affect the outcome and assay precision. To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed to add the TMB Chromogen Solution. A mild microbial contamination of reagents, especially of the conjugate, wash buffer and diluent. A mild contamination of the TMB Chromogen Solution with the Conjugate. Use a clean disposable pipette tip for each reagent. A void pipettes with metal parts. Containers and semi-automatic pipette tips used for the Conjugate and TMB can be reused provided they are thoroughly rinsed with deionized/distilled water and dried prior to and after each usage. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use high quality water. A void exposure of the reagents to excessive heat or sunlight during storage and incubation.

PREPARATION OF REAGENTS
Wash Solution
Dilute 1:10 with deionized/distilled water prior to use. If crystals are present, should be dissolved at 37°C before use. Dilute 100 mL of the Wash Concentrate into a clean container and dilute by adding 900 mL of deionized/distilled water. Mix thoroughly. The wash solution is stable for 5 days at room temperature and 2 weeks at 2-8°C when stored in a tightly sealed bottle.

Microtitration Strips:
Select the number of coated strips required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant pack. The pouch must be resed to protect from moisture.

Assay Procedure
All specimens and reagents to reach room temperature (~25°C) before use. Serum Samples and Controls should be assayed in duplicate.

1. Mark the microtitration strips to be used.
2. Dilute serum samples 1:101 distributing 10 µL of serum into 1 mL of Sample Diluent.
3. Pipette 100 µL of each diluted serum sample and ready to use controls to the appropriate wells. Leave one well for the blank, performed using 100 µL of the TMB-substrate at the substrate incubation step. Add 30µL Sorbent M only to the wells of diluted samples.
4. Incubate for 45 minutes at 37°C.
5. Aspirate and wash each well four (4) times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
6. O NTE: Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision. If a microplate washer is not available, (a) completely aspirate the liquid from each well, (b) dispense 300 µL of the Wash Solution into each well, and (c) repeat step (a) and (b) four times.
8. Incubate for 15 minutes at room temperature. Avoid exposure to direct sunlight.
9. Incubate 100 mL of Stopping Solution to each well using a dispenser.
10. Read the absorbance of the solution in the wells within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 600 or 620 nm.
<table>
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