Qualitative determination of Anti-Treponema pallidum antibodies
For “in vitro” use only
Store at 2- 8 ° C

INTRODUCTION
Syphilis is a venereal disease caused by infection with T. pallidum. The microorganism is transmitted by direct contact through a productive lesion. The incubation period is approximately 20 days and the disease undergoes 3 distinct phases with different symptoms. Anti-Treponeme antibodies appear during the first phase and can remain in 85-90 % of treated asymptomatic patients.

PROPOSED USE
The method is used for determining the presence of Anti-Treponeme Pallidum antibodies.

NECESSARY MATERIALS
“U”-shaped micro-titer plates.

FUNDAMENT OF THE METHOD
TPHA (Treponema Pallidum Hemagglutination) is an indirect hemagglutination assay carried out on microplates for the qualitative and semi-qualitative detection of anti-Treponema pallidum specific antibodies in human serum. Avian blood cells stabilized and sensitized with a solution of T. pallidum antigen agglutinate in the presence of anti-T Pallidum antibodies, exhibiting a typical agglutination pattern.

CONTENT

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<tbody>
<tr>
<td><strong>R1 Trial Cells</strong></td>
<td>Avian blood cells stabilized and sensitized with T. pallidum antigens (Nichols), sodium azide 0.95 g/l, and pH 7.2.</td>
</tr>
<tr>
<td><strong>R2 Control Cells</strong></td>
<td>Stabilized suspension of avian red blood cells, sodium azide 0.95 g/l pH 7.2.</td>
</tr>
<tr>
<td><strong>R3 Diluent</strong></td>
<td>Phosphate buffer, T. pallidum extract (Reiter), sodium azide 0.95 g/l, pH 7.2</td>
</tr>
<tr>
<td><strong>R4 Positive Control</strong></td>
<td>Pre-diluted human immune serum 1:20. sodium azide 0.95 g/l</td>
</tr>
<tr>
<td><strong>R5 Negative Control</strong></td>
<td>Rabbit serum, sodium azide 0.95 g/l</td>
</tr>
</tbody>
</table>
PRECAUTIONS
All components of human origin have tested negative to HBs, HCV and to anti-HIV (1/2) antigens, however they must be handled with caution as potentially infectious.

CALIBRATION
The sensitivity of the reagent can be traced to the 1st WHO International Syphilis Standard.

CONSERVATION AND STABILITY
All the components in the reagent kit will remain stable until the date of expiry indicated on the label of the flask provided they are kept airtight and stored at 2 - 8 °C, and care is exercised in order to avoid contamination during their use. Do not freeze the reagents as this will irreversibly modify their reactivity.

Always store the Trial Cells vials, R1 and R2 Control flasks in a vertical position. Storing vials in a horizontal position may cause the appearance of cell aggregates.

**Indicators of deterioration of the reagents:** presence of particle aggregates and turbidity.

SAMPLES
Fresh serum or plasma: will remain stable during a period of 7 days when stored at 2-8 °C or 3 months at -20 °C.

Samples with fibrin residues must be centrifuged before using. Do not use highly hemolyzed or lipemic samples.

PROCEDURE
Qualitative method:
1. Allow the reagents and samples to stand at room temperature.
2. Prepare a 1:20 dilution of the sample using the Diluent (10 µL serum + 190 µl Diluent).
3. Use a pipette to transfer the dilution to the adjacent wells of a micro-titration plate (Note 1):

<table>
<thead>
<tr>
<th>Sample 1/20 or Controls (µl)</th>
<th>25</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Cells (µl)</td>
<td>75</td>
<td>--</td>
</tr>
<tr>
<td>Trial Cells (µl)</td>
<td>--</td>
<td>75</td>
</tr>
</tbody>
</table>

4. Gently move the plate until the samples are completely homogenized.
5. Cover the plate and incubate at room temperature during 45-60 min (Note 2).
6. Macroscopically examine the agglutination patterns of the cells.

Semi-Qualitative Method
1. Prepare two dilutions of the sample with the Diluent, beginning with a dilution of 1:20.
2. Test each of the dilutions as described in the qualitative method.

READING AND INTERPRETATION

Results must be read by comparing the agglutination pattern of the trial cells with that of the Control Cells (Note 3). Results are assessed in accordance with the following criteria:

<table>
<thead>
<tr>
<th>Agglutination Degree</th>
<th>Reading</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Layer of smooth cells covering the entire bottom of the well, at times the borders draw up.</td>
<td>4+</td>
<td>Reactive</td>
</tr>
<tr>
<td>Cell layer covering part of the well bottom</td>
<td>3+</td>
<td>Reactive</td>
</tr>
<tr>
<td>Cell layer surrounded by a red circle</td>
<td>2+</td>
<td>Reactive</td>
</tr>
<tr>
<td>Cell layer covering less area and surrounded by a red circle.</td>
<td>1+</td>
<td>Reactive</td>
</tr>
<tr>
<td>Cell button with small orifice in the center.</td>
<td>±</td>
<td>Limit</td>
</tr>
<tr>
<td>Complete and well defined cell button, at times with a small orifice in the center.</td>
<td>-</td>
<td>Negative</td>
</tr>
</tbody>
</table>

The Negative Control must not exhibit agglutination when combined with the Trial Cells or with the Control Cells.

The Positive Control can only exhibit agglutination when combined with the Trial Cells. The presence of agglutination with the Control Cells indicates the presence of non-specific antibodies and must not be interpreted.

Samples with borderline results must be assayed again and interpreted as negative in the event of the same agglutination pattern.

Positive samples must be tittered as indicated in the semi-quantitative method. The titer is defined as the highest dilution with positive results.

Clinical diagnosis should not be based solely on the results of one assay. The prior history of the patient should also be considered.

QUALITY CONTROL
Use of both the positive and the negative controls is recommended in order to monitor the reactivity of the reagent and to use it as a comparison model for interpreting results.
CHARACTERISTICS OF THE METHOD

1. *Analytical sensitivity:* correct determination of the reference materials titer under the conditions described in the assay (see calibration).
2. *Prozone Effect:* Prozone effect is not observed until titers $\geq 1/163840$ (note 4).
3. *Diagnostic sensitivity:* 99.5 %
4. *Diagnostic specificity:* 100 %

INTERFERENCES

Do not interfere: Bilirubin (20 mg/L), hemoglobin (10 g/L), lipids (10 g/L) and rheumatoid factors (300 UI/mL). Other substances can interfere.

NOTES

1. Shake the vials with the Trial and Control Cells vigorously immediately before use.
2. Keep the microplates away from vibration and heat sources and also from direct sunlight.
3. The Control Cells agglutination pattern should not be used as a model for interpreting negative results as these cells produce more compact buttons than the Trial Cells.
4. Sera with elevated antibody titers can exhibit agglutination patterns with drawn-up borders.

LIMITATIONS OF THE METHOD

- The TPHA Assay presents certain non-specificities to antibodies from other treponeme pathogen species. All positive results should be confirmed with alternative methods such as FTA-Abs.
- False positive reactions have been described in samples from patients with mononucleosis, leprosy, borreliosis, autoimmune diseases and drug addiction.
- The TPHA Assay should not be used to control the efficacy of the treatment since antibodies level persists for a long time after the disease has been cured.

REFERENCE


PRESENTATION

Code: 1200408 100 tests: 7.5 ml Trial Cells : 7.5 ml Control Cells : 20 ml Diluent : 1 ml Positive Control : 1 ml Negative Control