Agglutination Assay -glass slide
For "in vitro" use only
Store at 2- 8 °C

INTRODUCRION
Syphilis is a sexually transmitted disease caused by Treponema pallidum. The main tests used to diagnose syphilis are: flocculation or agglutination, fluorescent antibody assays and hemagglutination assays.

PROPOSED USE
This method is used for determining plasma reagins in human sera.

MATERIALS
Micropipettes

FUNDAMENT OF THE METHOD
The method is based on the flocculation reaction of a stabilized suspension of cholesterol crystals, cardiolipin, lecithin and carbon particles in order to facilitate reading. This reaction behaves like an antigen to the antibodies present in individuals with syphilis. Said antibodies are called luetic reagins and their detection is part of a non-treponeme luetic serology.

CONTENT

<table>
<thead>
<tr>
<th>CONTENT</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1 Suspension White Cap</td>
<td>RPR-Carbon Suspension Phosphate tampon solution 10 mmol/l Sodium azide 0.95 g/l Code: 1200401: 1 x 3 ml Code: 1200402: 2 x 5 ml</td>
</tr>
<tr>
<td>R2 Positive Control Red Cap</td>
<td>Human serum with reagin titter ≥ 1/2. Sodium azide 0.95 g/l.</td>
</tr>
<tr>
<td>R3 Negative Control Blue Cap</td>
<td>Animal serum Sodium azide 0.95 g/l.</td>
</tr>
<tr>
<td>Glass slides</td>
<td>Code 1200401: 18 pack x 8 Code 1200402: 54 pack x 8</td>
</tr>
<tr>
<td>Flask and Dispensing Needle</td>
<td>1</td>
</tr>
</tbody>
</table>
CALIBRATION
The sensibility of the reagent can be traced to the CDC (Centre for Diseases Control) “Reference Human Serum” and comparable to the CDC “RPR-card test reagent”.

PREPARATION AND STABILITY
The R1 reagent is a suspension of carbon particles which should be homogenized before using by shaking gently, since sediment tends to form when the solution is allowed to stand.

Add 20 µl of R1 in each of the wells. Use a micropitte adjusted to this volume or adapt the needle provided with the kit to the plastic bottle. The solution in the bottle can be transferred by sucking- in through the needle. Any variation of the volume of the reagent can affect the results.

The reagents must be stored at 2-8 °C. Do not freeze. The components will remain active when stored under these conditions until the date of expiry indicated on the label.

SAMPLE
The sera will remain active for 7 days when stored at 2- 8 °C or for 3 months when stored at a -20 °C.

Do not use hemolyzed or contaminated sera. Do not use highly lipemic sera as this can cause non-specific agglutination. Samples with fibrin residues must be centrifuged before the assay.

PROCEDURE
Qualitative Method
1. - Allow the reagents and samples to reach room temperature as the sensitivity of the assay drops with low temperatures.
2. - Add 50 µl of the trial samples and a drop from each of the positive and negative controls onto the circles on the glass slide.
3. - Homogenize R1 by shaking gently. Invert the dispenser flask and gently press in order to eliminate air bubbles.
4. - Hold the micropipette in a vertical position- perpendicular to the glass slides- and add a drop (20 µl) of the carbon suspension next to the other drops.
5. - Mix the drops with an applicator, spread inside the circle. Use a different applicator for each sample.
6. - Place the glass slide in a rotatory stirrer (80-100 r.p.m.) for 8 minutes. Do not wait too long as this can cause false positive results.

Semi-quantitative Method
1. - Prepare two dilutions of the sample in a saline solution 9 g/l.
2. - Process each dilution as described above.
READING AND INTERPRETATION
Examine the preparation macroscopically looking for the presence or absence of agglutination immediately after withdrawing the glass slide from the stirrer.

**Interpretation**

<table>
<thead>
<tr>
<th>Type of Agglutination</th>
<th>Reading</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large or medium size aggregates</td>
<td>R</td>
<td>Reactive</td>
</tr>
<tr>
<td>Small aggregates</td>
<td>D</td>
<td>Mildly reactive</td>
</tr>
<tr>
<td>No aggregates or mild rough appearance</td>
<td>N</td>
<td>Does not react</td>
</tr>
</tbody>
</table>

In the semi-quantitative method, the titter is defined as the highest dilution with positive results.

**QUALITY CONTROL**

Users are advised to use both positive and negative controls in order to monitor the activity of the latex reagent and as a comparison model for interpreting results.
FUNCTIONAL CHARACTERISTICS
Detection Threshold: 1/16
Prozone Value: Negative up to 1/128
Diagnostic sensitivity: 86 % in primary syphilis and 100 % in secondary syphilis.
Diagnostic specificity: 98 %
Interference: Interferes with the rheumatoid factor starting at 300 UI/ml.
No interference: Bilirubin up to 342 µmoles/l
Hemoglobin up to 10 g/L
Lipids up to 10 g/L

LIMITATIONS OF THE METHOD
The RPR-Carbon test is not specific for diagnosing syphilis. All samples should be tested with treponemal assays such as TPHA and FTA-Abs for confirmation of results.
Negative result does not exclude a diagnosis of syphilis.
False positive results can occur with other diseases such as infectious mononucleosis, viral pneumonia, toxoplasmosis, pregnancy and autoimmune diseases.

PRECAUTIONS
The positive control is human serum. Although the presence of HB or anti-HIV antibodies has not been detected the preparation should be handled with the same precaution as the trial serum, in other words as potentially infectious. The reagents contain small amounts of sodium azide. Avoid contact with irritated skin and mucosa. Do not freeze.

WARNING
High environmental temperatures can cause the reaction sample to dry up on the glass slide with an aspect very similar to agglutination and therefore can be confused with a false positive. The trial should be carried out in a moisture chamber.

REFERENCES:

PRESENTATION
Code: 1200401 150 Det.: 3 mL suspension
: 0.8 ml Positive Control
: 0.8 ml Negative Control
: 18 x 8 glass slides.
: Flask and dispensing needle
Code: 1200402  500 Det.: 2 x 5 mL Suspension
       : 0.8 ml Positive Control
       : 0.8 ml Negative Control
       : 54 x 8 glass slides.
       : Flask and dispensing needle.
       : Applicator