Glass Slide Agglutination Assay
For "in vitro" use only
Store at 2- 8 °C

INTRODUCTION
Reagins are a group of antibodies directed against the very components of the body in patients suffering from Treponema Pallidum infection, the causal agent of syphilis. This microorganism causes lesions in the liver and the heart causing the release of small fragments of these organs into the blood stream which in turn are not recognized by the body. The patient’s immune system reacts with the formation of reagins, -antibodies against these fragments -.

This assay is useful for monitoring the patient’s response to treatment with antibiotics.

PROPOSED USE
For determining the presence of plasma reagins in human serum.

MATERIALS
Glass pipette 0.5 or 1 ml
Micropipettes
Rotatory mechanical stirrer (80 r.p.m)
Glass slides
Flat bottom glass flasks
Light microscope 10 xs
Glass tubes

FUNDAMENT OF THE METHOD
The VDRL Assay is a non-treponemic, glass slide agglutination procedure for the qualitative and semi-quantitative detection of plasma reagins. A suspension of antigens, a mixture of complex lipids, is agglutinated in the presence of reagins from the sample taken from a patient with syphilis.

CONTENT

<table>
<thead>
<tr>
<th></th>
<th>Code: 1200405: 6 x 0.5 ml</th>
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</thead>
<tbody>
<tr>
<td>R2 Antigen</td>
<td>Alcohol solution containing cardiolipin 0.3 g/l, lecithin 2.1 g/l and cholesterol 9 g/l.</td>
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<tr>
<td>Code: 1200407: 1 x 0.5 ml</td>
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<tr>
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<th>Code: 1200405: 1 x 0.5 ml</th>
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<tbody>
<tr>
<td>R3-PositiveControl Red Cap</td>
<td>Human serum with a reagins titer ≥ 1/2. sodium azide 0.95 g/l.</td>
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<td>Code: 1200406: 1 x 0.5 ml</td>
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<tr>
<td>R4NegativeControl Blue Cap</td>
<td>Animal serum, sodium azide 0.95 g/l.</td>
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CALIBRATION The sensibility of the reagent can be traced to “Reference Human Serum” of the CDC (Centre for Diseases Control) and comparable to the reagent “RPR-card test reagent” of the CDC.

PREPARATION
Antigen Suspension:
- Allow the VDRL antigen and VDRL Diluent to stand at room temperature (25 a 30 °C).

- Open the Diluent VDRL flask and transfer with a pipette 0.4 ml into a 25 ml flat bottom glass flash.

- Open the vial containing the antigen and use a glass pipette to add drop by drop, 0.5 ml of VDRL Antigen, on the VDRL Diluent, while turning the glass flask vigorously over a flat surface.

- Continue turning for 10 seconds more.

- Add 4.1 mL of VDRL Diluent allowing it to descend along the walls of the flask.

- Cap the flask and shake vertically approximately 30 times for 10 seconds.

- Allow the preparation to stand for 5 minutes. The antigen solution is now ready. Homogenize before using.

Note: Do not fraction the reagent. Follow instructions carefully when preparing the antigen suspension in order to achieve maximum results.
CONSERVATION AND STABILITY

All the components in the kit will remain stable until the date of expiry indicated on the label provided they are kept airtight and stored at 2-8 °C and care is exercise in order to avoid contamination while using.

VDRL ANTIGEN SUSPENSION: After preparation the suspension will remain stable for 24 hours at 15-25 °C. Do not freeze.

INDICATORS OF DETERIORATION OF THE REAGENTS: Presence of particles and turbidity.

SAMPLE

Fresh human serum: the sample will remain stable during 7 days at 2-8 °C or 3 months at -20 °C. The samples should be inactivated in glass tubes at 56 °C during 30 min. Special attention should be paid to inactivation conditions since too much time or heat may cause the sample to coagulate. Samples with fibrin residues must be centrifuged before the assay. Do not use hemolyzed or lipemic samples.

PROCEDURE

Qualitative method

- Allow the reagents and samples to reach room temperature. Sensitivity of the assay drops with low temperatures.
- Add 50 µl of sample and a drop from each of the positive and negative controls onto different circles in the glass slide.
- Gently homogenize the VDRL antigen suspension before using and add 1 drop (20 µl) onto each of the previous drops.
- Place the glass slide in a rotatory stirrer (160-180 r.p.m.) for 4 minutes. More time can give rise to false positives.

Semi-quantitative method

- Prepare two dilutions of the sample in 9 g/l saline solution. - Process each dilution as described in the qualitative assay.

READING AND INTERPRETATION

Examine immediately after shaking under a light microscope (10x) looking to the presence or absence of agglutination.

<table>
<thead>
<tr>
<th>Type of agglutination</th>
<th>Reading</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large or medium size aggregates - R</td>
<td>R</td>
<td>Reactive</td>
</tr>
<tr>
<td>Small aggregates - W</td>
<td>W</td>
<td>Weak</td>
</tr>
<tr>
<td>No or mildly rouge surface aggregates - N</td>
<td>N</td>
<td>Not Reactive</td>
</tr>
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</table>

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

CONTROL DE CALIDAD

Users are recommended to use the positive and negative controls for monitoring the functionality of the reagent and as a comparison model for interpreting results.

FUNCTIONAL CHARACTERISTICS

Prozone Effect: Not observed until titers ≥1/128.
Diagnostic sensitivity: 78 % (in primary syphilis) and 100% (in secondary syphilis).
Diagnostic Specificity: 98%.

INTERFERENCES

Bilirubin (20mg/L), hemoglobin (10g/L) and lipids (10g/L) do not interfere. Rheumatoid factors do interfere (300 UI/mL). Other substances can also interfere.

LIMITATIONS OF THE METHOD

The VDRL method is not specific for diagnosing syphilis. All samples should be tested with treponemic
assays such as TPHA and FTA-Abs for confirmation of results.

A 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. The reagents contain small amounts of sodium azide. Avoid contact with irritated skin and mucosa. Do not freeze.

REFERENCE


PRESENTATION

<table>
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<tr>
<th>Code</th>
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<tbody>
<tr>
<td>1200405</td>
<td>1500</td>
<td>1200406</td>
<td>250</td>
</tr>
<tr>
<td>1200407</td>
<td>150</td>
<td>1</td>
<td>0</td>
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</table>

5 ml Diluent
1 ml Control –
1 ml Control +

6 x 0,5 ml Antigen 1 x 055 mL Antigen
30 ml Diluent 5 mL Diluent
1 ml Control – 1 mL Control -
1 mL Control + 1 mL Control +

1 x 0.5 mL Antigen