SUMMARY
Syphilis is a venereal disease caused by Treponema pallidum, which invades intact mucous membranes or damaged skin areas. Sexual contact is the most common way of transmitting this disease. Microorganisms multiply and spread quickly after invasion. Illness' detection and treatment in early stages, are essential to avoid severe complications as neurosyphilis, cardiovascular syphilis and congenital syphilis. Disease's diagnosis has always been confronted with the difficulty to detect the etiological agent when skin lesions are not yet observed, as well as with the lack of culture methods for microorganisms' isolation. However, certain substances called "reagins" appear in the serum of an infected individual from the disease's onset and they react with cardiolipin/lecithin/cholesterol antigen. These reactions, together with clinical signs, are thus the quickest and more useful procedure available for syphilis diagnosis.

PRINCIPLE
Reagins present in individuals infected with T. pallidum are detected in serum by their reaction with a purified and stabilized cardiolipin antigen. If the sample contains reagins, they will bind to the antigen yielding a flocculation visible by microscope. Non-specific reactions are avoided using a highly purified antigen and adding choline chloride, which is distinctive of the USR (Unheated Serum Reagin) technique where inactivation of the sample is not necessary.

REQUIRED MATERIAL
1- Provided
-1 dropper

2- Non-Provided
- Rotating shaker adjustable at 180 rpm.
- Transparent glass slide with sections of approximately 14 mm each.
- Micropipettes for measuring the stated volumes.
- Microscope.

SAMPLE
Serum or cerebrospinal fluid
a) Collection: obtain in the usual way. Do not inactivate.
b) Additives: not required.
c) Known interfering substances: hemolysis or hyperlipemia may cause erroneous results.
d) Stability and storage instructions: in case they are not processed immediately, samples can be store for up to one week at 2-10°C.

PROCEDURE
Bring reagents and sample at room temperature before testing.

I- QUALITATIVE SLIDE TEST IN SERUM
In each section of a slide, place:

<table>
<thead>
<tr>
<th>Sample</th>
<th>50 ul</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A</td>
<td>1 drop</td>
</tr>
</tbody>
</table>

Rotate slide horizontally at 180 rpm during 4 minutes. Observe tests immediately after rotation, with a microscope (60 to 100 x).

II- SEMI-QUANTITATIVE SLIDE TEST IN SERUM
Prepare sample dilutions of 1:2; 1:4; 1:8; 1:16 and 1:32 with saline solution and complete test for each dilution as described in I).
III- QUALITATIVE TEST FOR CEREBROSPINAL FLUID

Dilute the Reagent A 1:2 with 10 g/dl sodium chloride solution. Use within the 2 hours of preparation.
In each section of a slide, place:

<table>
<thead>
<tr>
<th>Sample</th>
<th>50 ul</th>
</tr>
</thead>
<tbody>
<tr>
<td>With needle, caliber 6, add:</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diluted Reagent A</th>
<th>1 drop (10 ul)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mix thoroughly and rotate slide horizontally at 180 rpm during 8 minutes. Read tests immediately after rotation in a microscope (60 to 100 x).</td>
<td></td>
</tr>
</tbody>
</table>

INTERPRETATION OF RESULTS

Reactive: flocculation presence.
Non-reactive: complete absence of flocculation.
Semi-quantitative test: titer will be given as the inverse of the last dilution producing Reactive result. Read PROCEDURE LIMITATIONS carefully.

QUALITY CONTROL METHOD

In order to control system quality, process, as if it were samples, a Positive Control (serum known to be reactive) and a Negative Control (serum known to be non-reactive).

PROCEDURE LIMITATIONS

See Known Interfering Substances under SAMPLE.
Falsely positive results can be observed in individuals suffering from hepatitis, influenza, brucellosis, leprosy, malaria, asthma, tuberculosis, cancer, diabetes and autoimmune diseases.
These are not common cases and they generally show reactions with low titer and a medical record not coincident with syphilis symptoms.
For these reasons, it is absolutely necessary to perform a semi-quantitative test when a reactive qualitative test appears. Falsely negative results can be observed when a prozone phenomenon appears. For this reason, it is recommended to repeat test on serum diluted 1:5 with saline to verify results. If flocculation is observed under these conditions, sample is reactive.
In spite of the advantages of this method, the results obtained as well as those from any other serological methods must be considered only as a diagnostic aid, which must be checked against patient’s medical record.

PERFORMANCE

On 2,140 samples from a local hospital were assayed using V.D.R.L. test and immunofluorescence as a reference method. The concordance observed was higher than 96%.

WIENER LAB. PROVIDES

Kit for 250 tests (Cat. 1853151).

REFERENCES

Symbols

The following symbols are used in the packaging for Wiener lab. diagnostic reagent kits.

- **CE**
  - This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

- **IVD**
  - "In vitro" diagnostic medical device

- **Σ**
  - Contains sufficient for <n> tests

- **Use by**

- **Temperature limitation (store at)**

- **Do not freeze**

- **Biological risks**

- **Volume after reconstitution**

- **Contents**

- **Batch code**

- **Manufactured by:**

- **Harmful**

- **Corrosive / Caustic**

- **Irritant**

- **Consult instructions for use**

- **Calibrator**

- **Control**

- **Positive Control**

- **Negative Control**

- **Catalog number**