INTRODUCTION:
Syphilis is still a common sexually transmitted disease in many areas of the world. In 1999 the WHO estimated that the worldwide annual incidence of sexually acquired syphilis was 12 million cases. Venereal syphilis is divided into: early syphilis subdivided into primary, secondary and early latent stages; late syphilis that may occur after extended periods of latent syphilis. Serological tests for syphilis are subdivided into: non treponemal tests that measure IgM and IgG antibodies to lipoidal material released from damaged host cells and antibodies to lipoprotein-like material and cardiolipin released from the treponemes. The most commonly used are RPR card and VDRL. The tests are used for screening and for determining the efficacy of treatment. They lack sensitivity in early primary syphilis and in late syphilis and it can appear a prozone reaction or false positive results. Treponema tests use T. pallidum subsp. pallidum or its derivatives (recombinant proteins). They are used as confirmatory tests and in establishing the diagnosis of late latent or late syphilis. The most commonly used tests are: FTA-ABS, TP-PA (T. pallidum particle agglutination) and MHA-IP (micro hemagglutination assay to T. pallidum). Several tests using enzyme immunoassays (EIA) have been used as confirmatory test for syphilis. They have sensitivities and specificities similar to those of the other treponemal tests.

PRINCIPLE OF THE TEST:
The ELISA method is based upon the reaction of antibodies in the sample tested with the antigen adsorbed on the polystyrene surface. Unbound immunoglobulins are washed off. An enzyme-labelled anti-human globulin binds the antigen-antibody complex in a second step. After a new washing step, bound conjugate is developed with the aid of a substrate solution (TMB) to render a blue coloured soluble product which turns into yellow after adding the acid stopping solution.

KIT FEATURES:
All reagents, except for the washing solution, are supplied ready to use. Serum dilution solution and conjugate are coloured to help in the performance of the technique. Sample predilution is not necessary. Break-apart individual wells are supplied, so that the same number of wells is consumed than the number of tests performed.

KIT CONTENTS:
- VIRCELL SYPHILIS PLATE: 1 96-wells plate coated with antigen of T. pallidum.

STABILITY AND HANDLING OF REAGENTS:
Handle reagents in aseptic conditions to avoid microbial contaminations. Do not let the plate dry between washing and reagent addition. Substrate solution is light sensitive. Avoid light exposure and discard if blue colour develops during storage. Substrate solution should not get in contact with oxidizers such as bleach solutions or metals. Make sure that no metal components come in contact with the substrate. Use only the amount of washing, serum dilution, conjugate and TMB solutions required for the test. Do not return the excess solution into the bottles.

VIRCELL, S.L. does not accept responsibility for the mishandling of the reagents included in the kit.

RECOMMENDATIONS AND PRECAUTIONS:
1. For in vitro diagnosis use only. For professional use only.
2. Use kit components only. Do not mix components from different kits or manufacturers. Only the serum dilution,
washing, stopping and substrate solutions are compatible with the equivalents in other VIRCELL ELISA references and lots. 
3. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material. 
4. Do not use in the event of damage to the package. 
5. Never pipette by mouth. 
6. Serum dilution solution, plate, conjugates and controls in this kit include substances of animal origin. Controls include as well substances of human origin. Although the human serum controls of this kit have been tested and found negative for Hepatitis B Surface Antigen (HBsAg), Hepatitis C antibodies and Human Immunodeficiency Virus antibodies, control sera and patient specimens should be handled as potentially infectious. 
The wells are coated with inactivated T. pallidum antigen. Nevertheless, they should be considered potentially infectious and handled with care. No present method can offer complete assurance that these or other infectious agents are absent. 
All material should be handled and disposed as potentially infectious. Observe the local regulations for clinical waste disposal. 
7. Substrate solution may be irritant to skin and mucus. In case of contact with this solution, rinse thoroughly with water and seek medical attention. For further information a Material Safety Data Sheet is available. 
8. Before incorporating this product onto an automatic processing system, we strongly recommend the performance of a pre-evaluation assay. To this purpose, VIRCELL counts with sets of samples reserved for evaluation in parallel with the manual technique. These sets of samples are available on request, as well as a list of commercial systems which have already been validated for use with the VIRCELL ELISA range. 
9. During incubation times, an adequate sealing of the plates with the adhesive film included in the kit avoids the desiccation of the samples, and guarantees the repeatability of the results. 

SPECIMEN COLLECTION AND HANDLING: 
Blood should be collected aseptically using venipuncture techniques by qualified personnel. Use of sterile or aseptic techniques will preserve the integrity of the specimen. Serum/plasma samples are to be refrigerated (2-8°C) upon collection or frozen (-20°C) if the test cannot be performed within 7 days. Samples should not be repeatedly frozen and thawed. Do not use hyperlipemic, hemolysed or contaminated sera. Samples containing particles should be clarified by centrifugation. The kit is suitable for use with serum or plasma (collected with EDTA or citrate). 

PRELIMINARY PREPARATION OF THE REAGENTS: 
Only the washing solution must be prepared in advance. Fill 50 ml of 20x washing solution up to 1 litre with distilled water. 
Should salt crystals form in the washing concentrate during storage, warm the solution to 37°C before diluting. Once diluted, store at 2-8°C. 

ASSAY PROCEDURE: 
1. Set incubator/ water bath to 37±1°C. 
2. Bring all reagents to room temperature before use (approx. 1 hour), without removing the plate from the bag. 
3. Shake all components. 
4. Remove the plate from the package. Determine the numbers of wells to be employed counting in four wells for the controls: two for the cut off serum and one each for the negative and positive sera. Wells not required for the test should be returned to the pouch, which should then be sealed. 
5. Add 100 µl of serum dilution solution 3 to all wells. Add 5 µl of each sample, 5 µl of positive control 3, 5 µl of cut off serum 3 (cut off serum in duplicate) and 5 µl of negative control 3 into the corresponding wells. If the assay is performed manually, shake the plate in a plate shaker (2 min) in order to achieve a homogenous mixture of the reagents. If for some reason correct shaking cannot be guaranteed, a pre-dilution of the sample in a separate tube or plate should be made, using double volume of serum diluent 3 and sample. Mix homogenously with the pipette and dispense 105 µl of each diluted sample to the wells 3. 
6. Cover with a sealing sheet and incubate at 37±1°C for 45 min. 
7. Remove the seal, aspirate liquid from all wells and wash five times with 0.3 ml of washing solution 3 per well. Drain off any remaining liquid. 
8. Immediately add 100 µl of conjugate solution 3 into each well. 
9. Cover with a sealing sheet and incubate in incubator/ water bath at 37±1°C for 30 min. 
10. Remove the seal, aspirate liquid from all wells and wash five times with 0.3 ml of washing solution 3 per well. Drain off any remaining liquid. 
11. Immediately add 100 µl of substrate solution 3 into each well. 
12. Incubate at room temperature for 20 min protected from light. 
13. Add immediately 50 µl of stopping solution 3 into all wells. 
14. Read with an spectrophotometer at 450/620 nm within 1 hour of stopping. 

INTERNAL QUALITY CONTROL: 
Each batch is subjected to internal quality control (Q.C.) testing before batch release complying with specifications stricter than validation protocol for users. Final Q.C. results for each particular lot are available. 
The control material is traceable to reference sera panels internally validated. 

VALIDATION PROTOCOL FOR USERS: 
Positive, negative and cut off controls must be run with each test run. It allows the validation of the assay and kit. 
Optical densities (O.D.) must fall in the following ranges. 
Otherwise, the test is invalid and must be repeated. 

<table>
<thead>
<tr>
<th>Control</th>
<th>O.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>&gt;0.9</td>
</tr>
<tr>
<td>Negative control</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Cut off control</td>
<td>&gt;0.55</td>
</tr>
<tr>
<td></td>
<td>&lt;1.5</td>
</tr>
</tbody>
</table>

INTERPRETATION OF RESULTS: 
Calculate the mean O.D. for cut off serum. 

Antibody index=[sample O.D./ cut off serum mean O.D.] x 10
Samples with equivocal results must be retested and/or a new sample obtained for confirmation. Samples with indexes below 9 are considered as not having antibodies against *T. pallidum*. Samples with indexes above 11 are considered as having antibodies against *T. pallidum*.

**LIMITATIONS:**
1. This kit is intended to be used with human serum/plasma.
2. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.
3. The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures. A definitive diagnosis should be made by isolation techniques.
4. This test will not indicate the site of infection. It is not intended to replace isolation.
5. Lack of significant rise in antibody level does not exclude the possibility of infection.
6. Samples collected very early in the course of an infection may not have detectable levels of IgG. In such cases, it is recommended an IgM assay be performed or a second serum/plasma sample be obtained 14 to 21 days later to be tested in parallel with the original sample to determine seroconversion.
7. Results in IgG detection in neonates must be interpreted with caution, since maternal IgG is transferred passively from the mother to the foetus before birth. IgM assays are generally more useful indicators of infection in children below 6 months of age.
8. The results of a single-specimen antibody determination should not be used to aid in the diagnosis of recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to look for seroconversion or a significant rise in antibody level.

**PERFORMANCE:**
• **SENSITIVITY AND SPECIFICITY:**
  296 serum/plasma samples from donors were assayed with SYPHILIS ELISA IgG+IgM against another commercial ELISA kit. The results were as follows:

<table>
<thead>
<tr>
<th>Index</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 9</td>
<td>Negative</td>
</tr>
<tr>
<td>9-11</td>
<td>Equivocal</td>
</tr>
<tr>
<td>&gt;11</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Indeterminate values were omitted from the final calculations.

• **INTRA-ASSAY PRECISION:**
  3 sera were individually pipetted 10 times each serum in a single assay performed by the same operator in essentially unchanged conditions. The results were as follows:

<table>
<thead>
<tr>
<th>Serum</th>
<th>N</th>
<th>%C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>10</td>
<td>1.33</td>
</tr>
<tr>
<td>NC</td>
<td>10</td>
<td>3.38</td>
</tr>
<tr>
<td>CO</td>
<td>10</td>
<td>6.73</td>
</tr>
</tbody>
</table>

C.V. Coefficient of variation

• **INTER-ASSAY PRECISION:**
  3 sera were individually pipetted on 5 consecutive days by 2 different operators. The results were as follows:

<table>
<thead>
<tr>
<th>Serum</th>
<th>N</th>
<th>%C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>10</td>
<td>3.34</td>
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<td>CO</td>
<td>10</td>
<td>6.73</td>
</tr>
</tbody>
</table>

C.V. Coefficient of variation

• **CROSS REACTIVITY AND INTERFERENCES:**
  8 samples known to be positive for other spirochetes (*Borrelia burgdorferi*) and members of the symbiotic group (Herpes simplex 2, *Chlamydia trachomatis*) were assayed. 2 samples known to be positive for antinuclear antibodies were assayed for IgG+IgM testing. 2 samples known to be positive for rheumatoid factor were assayed for IgG+IgM testing.

The results of the test demonstrated the specific reaction of the kit with no cross reaction or interferences with the referred specimens.

**SYMBOLS USED IN LABELS:**
- **IVD** In vitro diagnostic medical device
- **Use by (expiration date)**
- **Store at x-ºC**
- **Contains sufficient for <X> test**
- **LOT** Batch code
- **REF** Catalogue number
- **Consult instructions for use**

**LITERATURE:**

various

Clin antibody immunoassay 9.

Importance antigens

Microbiol Immun 8.

immunofluorescence 10.

immunoglobulins testing.

Dis 6.

immunoglobulin 1989.

antigens


For any question please contact: customerservice@vircell.com

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