Treponema pallidum Screen ELISA (recombinant) with polyvalent IgG+M conjugate
IgG+M Test Kit

Order No.: EC150.00
Color Coding: pink metallic/black

FOR IN VITRO DIAGNOSIS ONLY

Sekisui Virotech GmbH
Löwenplatz 5
65428 Rüsselsheim / Germany

Tel.: +49-6142-6909-0
Fax: +49-6142-966613
http://www.sekisui_virotech.com
## Contents

1. Intended Use ........................................................................................................... 3
2. Diagnostic Relevance............................................................................................. 3
3. Test Principle............................................................................................................ 3
4. Package Contents (IgG+M Testkit) ........................................................................ 3
5. Storage and Shelflife of the Testkit and the ready to use reagents ....................... 4
6. Precautions and Warnings ...................................................................................... 4
7. Material required but not supplied ......................................................................... 4
8. Test Procedure ....................................................................................................... 4
   8.1 Examination Material .......................................................................................... 4
   8.2 Preparation of Reagents .................................................................................... 5
   8.3 Virotech ELISA Test Procedure............................................................................ 5
   8.4 Usage of ELISA processors .............................................................................. 5
9. Test Evaluation ....................................................................................................... 6
   9.1 Test function control .......................................................................................... 6
   9.2 Calculation of the Virotech Units (VE) ................................................................. 6
   9.3 Interpretation Scheme IgG/M .............................................................................. 6
   9.4 Limits of the Test .............................................................................................. 6
10. Performance Data .................................................................................................. 7
    10.1 Analytical Sensitivity and Specificity ................................................................. 7
    10.2 Cross-reactivity .............................................................................................. 7
    10.3 Prevalence (expected values) .......................................................................... 7
    10.4 Intra-Assay Coefficient of Variation (Repeatability) .......................................... 7
    10.5 Inter-Assay Coefficient of Variation (Reproducibility) ....................................... 8
11. Literature ................................................................................................................. 8
12. Test Procedure Scheme ........................................................................................ 9
1. **Intended Use**

The Treponema pallidum Screen ELISA is used for the simultaneous semiquantitative and qualitative determination of IgG and IgM antibodies (together) against Treponema pallidum in human serum.

2. **Diagnostic Relevance**

The genus *Treponema* includes several species and subspecies which are pathogenic in humans. *Treponema pallidum* subsp. pallidum is the pathogen of syphilis, a disease which only occurs in humans. Syphilis is generally transmitted sexually and normally occurs in three stages: primary stage, secondary stage and tertiary stage, each with latent or inactive phases (2). In addition, *T. pallidum* can be transmitted during pregnancy from the infected mother to the foetus (congenital syphilis) (2). The diagnosis depends on serological analysis, as *T. pallidum* cannot be cultured in vitro (1).

Serology provides decisive diagnostic information for patients with clinical symptoms indicating syphilis or whose medical history suggests syphilis. The exclusion diagnosis of syphilis is often needed for other patients with uncharacteristic symptoms. This may be justified, as the clinical course of this infection is often untypical (4).

*T. pallidum* infections induce two groups of antibodies in the host:

a) *Treponema* non-specific antibody, called reagin

b) *Treponema* specific antibodies, which react with *T. pallidum* and related strains.

The following diagnostic steps are required for reliable syphilis diagnosis (4):

1. **Screening test:** ELISA (polyvalent) or TPHA- / TPPA test
2. **Confirmatory test:** Immunoblot or FTA-ABS test (polyvalent)
3. **Evaluation of the activity of the infection:** 19S-IgM-FTA-ABS (IgM-ELISA) or VDRL test

Within this three step diagnostic scheme, the Treponema pallidum Screen ELISA can be used as a serological screening test, equivalent to the TPPA/TPHA. The ELISA uses recombinant antigens of *T. pallidum* (Tp47, Tp17 and Tp15).

After a positive or borderline screening test, a general distinction should be made between IgG- and IgM-specific *Treponema* antibodies. IgM usually indicates a fresh and active infection, while IgG is an indicator of past infection. Moreover, IgM activity in neonates indicates congenital syphilis (3).

For differentiation between the Ig classes and for simultaneous confirmation, we recommend the Virotech Treponema pallidum LINE Immunoblot (Order no. IgG WE150G32 and IgM WE150M32).

3. **Test Principle**

The antibody searched for in the human serum forms an immune complex with the antigen coated on the microtiter-plate. Unbound immunoglobulins are removed by washing processes. The enzyme conjugate attaches to this complex. Unbound conjugate is again removed by washing processes. After adding the substrate solution (TMB), a blue dye is produced by the bound enzyme (peroxidase). The color changes to yellow when the stopping solution is added.

4. **Package Contents (IgG+M Testkit)**

1. **1 Microtiter-Plate** consisting of 96 wells, freeze-dried
2. **PBS-Dilution Buffer (blue, ready to use) 2x50ml,** pH 7.2, with preservative and Tween 20
3. **PBS-Washing Solution (20x concentrated) 50ml,** pH 7.2, with preservative and Tween 20
4. **IgG+M negative Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
5. **IgG+M cut-off Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
6. **IgG+M positive Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
7. **IgG+M mixed conjugate (anti-human polyvalent IgG+IgM conjugate), 11ml,** (sheep or goat)-horseradish peroxidase-conjugate with protein stabilisers and preservative in Tris buffer, ready to use
8. **Tetramethylbenzidine substrate solution (3,3',5,5'-TMB), 11ml,** ready to use
9. **Citrate-Stopping Solution, 6ml,** contains an acid mixture
5. **Storage and Shelflife of the Testkit and the ready to use reagents**

Store the testkit at 2-8°C. The shelf life of all components is shown on each respective label; for the kit shelf life please see Quality Control Certificate.

1. Microtitre strips/single wells are to be resealed in package after taking out single wells and stored with desiccant at 2-8°C. Reagents should immediately be returned to storage at 2-8°C after usage.
2. The ready to use conjugate and the TMB-substrate solution are sensitive to light and have to be stored in dark. Should there be a color reaction of the substrate dilution due to incidence of light, it is not useable anymore.
3. Take out only the amount of ready to use conjugate or TMB needed for the test insertion. Additional conjugate or TMB taken out may not be returned but must be dismissed.

<table>
<thead>
<tr>
<th>Material</th>
<th>Status</th>
<th>Storage</th>
<th>Shelflife</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Samples</td>
<td>Diluted</td>
<td>+2 to +8°C</td>
<td>max. 6h</td>
</tr>
<tr>
<td>Controls</td>
<td>Undiluted</td>
<td>+2 to +8°C</td>
<td>1 week</td>
</tr>
<tr>
<td></td>
<td>After Opening</td>
<td>+2 to +8°C</td>
<td></td>
</tr>
<tr>
<td>Microtitreplate</td>
<td>After Opening</td>
<td>+2 to +8°C (storage in the provided bag with desiccant bag)</td>
<td>3 months</td>
</tr>
<tr>
<td>Rheumatoid factor -</td>
<td>Undiluted, After</td>
<td>+2 to +8°C</td>
<td>3 months</td>
</tr>
<tr>
<td>Absorbent</td>
<td>Opening</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conjugate</td>
<td>Diluted</td>
<td>+2 to +8°C</td>
<td>1 week</td>
</tr>
<tr>
<td>Tetramethylbenzidine</td>
<td>After Opening</td>
<td>+2 to +8°C (protect from light)</td>
<td>3 months</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>After Opening</td>
<td>+2 to +8°C</td>
<td>3 months</td>
</tr>
<tr>
<td>Washing Solution</td>
<td>Final Dilution (ready-to-use)</td>
<td>+2 to +25°C</td>
<td>4 weeks</td>
</tr>
</tbody>
</table>

6. **Precautions and Warnings**

1. Only sera which have been tested and found to be negative for HIV-1 antibodies, HIV-2 antibodies, HCV antibodies and Hepatitis-B surface-antigen are used as control sera. Nevertheless, samples, diluted samples, controls, conjugates and microtitre strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.
2. Those components that contain preservatives, the Citrate Stopping Solution and the TMB have an irritating effect to skin, eyes and mucous. If body parts are contacted, immediately wash them under flowing water and possibly consult a doctor.
3. The disposal of the used materials has to be done according to the country-specific guidelines.

7. **Material required but not supplied**

1. Aqua dest./demin.
2. Eight-channel pipette 50µl, 100µl
3. Micropipettes: 10µl, 100µl, 1000µl
4. Test tubes
5. Paper towels or absorbent paper
6. Cover for ELISA-plates
7. Disposal box for infectious material
8. ELISA handwasher or automated EIA plate washing device
9. ELISA plate spectrophotometer, wavelength = 450nm, reference length = 620nm (Reference Wavelength 620-690nm)
10. Incubator

8. **Test Procedure**

Working exactly referring to the Sekisui Virotech user manual is the prerequisite for obtaining correct results.

8.1 **Examination Material**

Either serum or plasma can be used as test material, even if only serum is mentioned in the instructions. Any type of anticoagulant can be used for plasma. Always prepare patient-dilution freshly.
For a longer storage the sera must be frozen. Repeated defrosting should be avoided.
1. Only fresh non-inactivated sera should be used.
2. Hyperlipaemic, haemolytic, microbially contaminated and turbid sera should not to be used (false positive/negative results).

8.2 Preparation of Reagents
The Sekisui Virotech System Diagnostic Testing offers a high degree of flexibility, as the dilution and washing buffers, TMB and citrate stop solution can be used for different parameters and batches. The ready to use controls (positive control, negative control, cut-off control) are **parameter specific** and **only to use** with the plate lot indicated in the Quality Control Certificate.

**Note:** The Treponema pallidum Screen ELISA conjugate can only be used for the Treponema pallidum Screen ELISA and is batch-specific!

1. Set incubator to 37°C and check proper temperature setting before start of incubation.
2. Bring all reagents to room temperature before opening package of microtiter strips.
3. Shake all liquid components well before use.
4. Make up the washing solution concentrate to 1 L with distilled or demineralized water. If crystals have formed in the concentrate, please bring the concentrate to room temperature before use and shake well before use.

8.3 Virotech ELISA Test Procedure
1. For each test run, pipette 100µl each of ready to use dilution buffer (blank), IgG+M-positive, negative and cut-off controls as well as diluted patient sera. We propose a double insertion (blank, controls and patient sera); for cut-off control a double insertion is absolutely necessary. Working dilution of patient sera: 1+100; e.g. 10µl serum + 1ml dilution buffer.
2. After pipetting start incubation for 30 min. at 37°C (with cover).
3. End incubation period by washing microtiter strips 4 times with 350 – 400µl washing solution per well. Do not leave any washing solution in the wells. Remove residues on a cellulose pad.
4. Pipette 100µl of ready to use conjugate into each well.
5. Incubation of conjugates: 30 min. at 37°C (with cover).
6. Stop conjugate incubation by washing 4 times (pls. refer to point 3 above).
7. Pipette 100µl of ready to use TMB into each well.
8. Incubation of substrate solution: 30 min. at 37°C (with cover, keep in dark).
9. Stopping of substrate reaction: pipette 50µl of citrate stopping solution into each well. Shake plate carefully and thoroughly until liquid is completely mixed and a homogeneous yellow color is visible.
10. Measure extinction (OD) at 450/620nm (Reference Wavelength 620-690nm). Set your photometer in such a way that the blank value is deducted from all other extinctions. Extinctions should be measured within 1 hour after adding the stopping solution!

Pls. refer to last page for Test Procedure Scheme

8.4 Usage of ELISA processors
All Sekisui Virotech ELISAs can be used on ELISA processors. The user is bound to proceed a validation of the devices (processors) on a regular basis.

Sekisui Virotech recommends the following procedure:
1. Sekisui Virotech recommends to proceed the validation of device referring to the instructions of the device manufacturer during the implementation of the ELISA processor respectively after bigger reparations.
2. It is recommended to check the ELISA-processor with the Validationkit (EC250.00) afterw ards. A regular check using the Validationkit shall be proceeded minimum once a quarter to test the accuracy of the processor.
3. The release criteria of the Quality Control Certificate of the product must be fulfilled for each testrun.

With this procedure, your ELISA processor will function properly and this will support quality assurance in your laboratory.
9. Test Evaluation

The ready to use controls serve for a semiquantitative determination of specific IgG+M-antibodies. Their concentration can be expressed in Virotech units = VE. Fluctuations resulting from the test procedure can be balanced with this calculation method and a high reproducibility is achieved in this way. Use the means of the OD values for calculation of the VE.

9.1 Test function control

a) OD-values
The OD of the blank should be < 0.15.
The OD-values of the negative controls should be lower than the OD-values mentioned in the Quality Control Certificate. The OD-values of the positive controls as well as of the cut-off controls should be above the OD-values mentioned in the Quality Control Certificate.

b) Virotech Units (VE)
The Virotech Units (VE) of the cut-off controls are defined as 10 VE. The calculated VE of the positive controls should be within the ranges mentioned in the Quality Control Certificate.
If those requirements (OD-values, VE) are not fulfilled, the test has to be repeated.

9.2 Calculation of the Virotech Units (VE)
The extinction of the blank value (450/620nm) has to be subtracted from all other extinctions.

\[
\begin{align*}
VE \text{ (positive control)} &= \frac{\text{OD (positive control)}}{\text{OD (cut-off control)}} \times 10 \\
VE \text{ (patient serum)} &= \frac{\text{OD (patient serum)}}{\text{OD (cut-off control)}} \times 10
\end{align*}
\]

9.3 Interpretation Scheme IgG+M

<table>
<thead>
<tr>
<th>Result (VE)</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 9.0</td>
<td>negative</td>
</tr>
<tr>
<td>9.0 - 11.0</td>
<td>borderline</td>
</tr>
<tr>
<td>&gt; 11.0</td>
<td>positive</td>
</tr>
</tbody>
</table>

1. If the measured values are above the defined borderline range, they are considered to be positive.
2. If the measured VE is within the borderline range, no significant high antibody concentration is present, the samples are considered to be borderline. For the secure detection of an infection it is necessary to determine the antibody concentration of two serum samples. One sample shall be taken directly at the beginning of the infection and a second sample 5 – 10 days later (convalescent serum). The antibody concentration of both samples has to be tested in parallel, that means in one test run. A correct diagnosis based on the evaluation of a single serum sample is not possible.
3. If the measured values are below the defined borderline range, no measurable antigen specific antibodies are present in the samples. The samples are considered to be negative.

9.4 Limits of the Test

1. The interpretation of serological results shall always include the clinical picture, epidemiological data and all further available laboratory results.
2. A negative result does not completely exclude the possibility of an infection with Treponema pallidum. The sample may have been taken before antibodies were formed, or the antibodies might lie under the limit of detection of the test.
3. No diagnostic statement can be made about neurosyphilis or neonatal syphilis, as no suitable serum or CSF samples were available for the evaluation.
4. Because of the high DNA homology between T. pallidum subsp. pallidum (syphilis), endemicum (endemic syphilis) and pertenue (yaws), and to some extent also Treponema carateum (pinta), cross-reactions must be expected. This means that serological tests cannot be used for the differential diagnosis of non-venereal treponematoses (4).
5. There may be non-specific reactions with HIV infections.
6. In some patients with latent syphilis, discrepancies have been observed between the 19S-IgM-FTAabs, Immunoblot and ELISA test results. The reason for this is unclear.

7. When interpreting isolated borderline or positive IgM results in pregnant women, the possibility must be born in mind that multireactive IgM antibodies are present. These results must be clarified with additional tests - 19S-IgM-FTA-ABS (IgM-ELISA) or the VDRL test. See “Diagnostic Significance”.

10. **Performance Data**

10.1 **Analytical Sensitivity and Specificity**

A total of 717 sera were tested in the TP Screen ELISA and evaluated in comparison to the overall result. The group of sera consists of 80 blood donor sera, 160 sera from pregnant women, 43 sera from prostitutes, 100 potentially cross-reacting sera, 63 HIV-positive sera, 21 sera from interlaboratory comparisons and 250 syphilis sera. The overall result is based on results obtained with the following tests: VDRL, RPR, KBR, TPPA, FTA-ABS, ELISA and Immunoblot.

<table>
<thead>
<tr>
<th>Group of Sera (n=717)</th>
<th>Treponema pallidum Screen ELISA polyvalent IgG+M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Reference Result</td>
<td>Negative</td>
</tr>
<tr>
<td>Negative</td>
<td>426</td>
</tr>
<tr>
<td>Borderline</td>
<td>5</td>
</tr>
<tr>
<td>Positive</td>
<td>0</td>
</tr>
</tbody>
</table>

This gives an analytical sensitivity of >99.9% and an analytical specificity of 95.9%.
Borderline results were excluded from the calculation.

10.2 **Cross-reactivity**

100 potentially cross-reactive sera (60 borreliosis, measles parvovirus, EBV and VZV sera) were used to test the cross-reactivity.

<table>
<thead>
<tr>
<th>n=100</th>
<th>IgG+M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>95</td>
</tr>
<tr>
<td>Borderline</td>
<td>3</td>
</tr>
<tr>
<td>Positive</td>
<td>2</td>
</tr>
</tbody>
</table>

The specificity is 97.9%.

10.3 **Prevalence (expected values)**

To determine the prevalence, 80 blood donor sera and 160 pregnancy sera were tested.

<table>
<thead>
<tr>
<th>n=240</th>
<th>IgG+M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>237</td>
</tr>
<tr>
<td>Borderline</td>
<td>2</td>
</tr>
<tr>
<td>Positive</td>
<td>1</td>
</tr>
</tbody>
</table>

This corresponds to a prevalence (borderline and positive) of 1.25%.
The specificity is 99.6%.

10.4 **Intra-Assay Coefficient of Variation (Repeatability)**

The strips from different plates in a batch were tested with a serum in a single assay. This gave a coefficient of variation of < 9%.
10.5 Inter-Assay Coefficient of Variation (Reproducibility)

3 sera were tested in 12 independent assays in different test laboratories and from different test persons. This gave a coefficient of variation of <15 %.

11. Literature

12. Test Procedure Scheme

Preparation of Patient Samples and Washing Solution

▼ Washing Solution: Fill up concentrate to 1 liter with aqua dest./demin.

IgG+M- Samples – Dilution
1:101
e.g.:
10 µl serum/plasma + 1000 µl Dilution Buffer
(Serum Dilution Buffer is ready to use)

Test procedure

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Temperature</th>
<th>Quantity</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Samples Incubation</strong></td>
<td>30 minutes</td>
<td>100 µl Patient Samples</td>
<td>30 minutes at 37°C</td>
</tr>
<tr>
<td><strong>Wash 4times</strong></td>
<td></td>
<td>400 µl Washing Solution</td>
<td>Remove Residues on a Cellulose Pad</td>
</tr>
<tr>
<td><strong>Conjugate Incubation</strong></td>
<td>30 minutes</td>
<td>100 µl Conjugate</td>
<td>30 minutes at 37°C</td>
</tr>
<tr>
<td><strong>Wash 4times</strong></td>
<td></td>
<td>400 µl Washing Solution</td>
<td>Remove Residues on a Cellulose Pad</td>
</tr>
<tr>
<td><strong>Substrate Incubation</strong></td>
<td>30 minutes</td>
<td>100 µl Substrate</td>
<td>30 minutes at 37°C</td>
</tr>
<tr>
<td><strong>Stopping</strong></td>
<td></td>
<td>50 µl Stopping Solution</td>
<td>shake carefully</td>
</tr>
<tr>
<td><strong>Measure Extinctions</strong></td>
<td></td>
<td>Photometer at 450/620nm</td>
<td>(Reference Wavelength 620-690nm)</td>
</tr>
</tbody>
</table>