Treponema pallidum LINE (recombinant)
IgG/IgM Line Immunoblot

Order No.:
WE150G16: IgG Line Immunoblot, 16 strips
WE150G32: IgG Line Immunoblot, 32 strips
WE150M16: IgM Line Immunoblot, 16 strips
WE150M32: IgM Line Immunoblot, 32 strips

FOR IN VITRO DIAGNOSIS ONLY

Virotech Diagnostics GmbH
Löwenplatz 5
D- 65428 Rüsselsheim
Tel.: +49-6142-6909-0
Fax: +49-6142-966613
http://www.virotechdiagnostics.com
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1. **Intended Use**

Line Immunoblot Testkit for the qualitative detection of *Treponema pallidum* specific IgG- respectively IgM- antibodies in human serum. The Kit can be used as confirmation test for an extended Syphilis diagnostic, in case the result of the screening test is doubtful (suspicious) or positive.

2. **Diagnostic Meaning**

The genus *Treponema pallidum* comprises several human pathogen species and subspecies. *Treponema pallidum* subsp. pallidum is the causative agent of syphilis (Lues), a disease occurring only in humans. Syphilis is generally transmitted sexually. Its natural course presents in three stages: primary, secondary, and tertiary syphilis, with periods of latency or inactive disease (2). Additionally, *T. pallidum* can be transmitted to the foetus from an infected mother during pregnancy (congenital syphilis) (2). As *T. pallidum* cannot be cultured in vitro (1), diagnosis depends on serological analysis.

Infection with *T. pallidum* provoke two groups of antibody development in the host:

a) Non-treponemal antibodies, called reagin
b) Treponemal specific antibodies, which react with *T. pallidum* and related strains.

For a good Syphilis-Diagnostic the following step-diagnostic is recommended (4):

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

It is desirable to differentiate between IgG and IgM-specific Treponema-antibodies. IgM, as a rule, shows an active infection whilst IgG is an indicator for a recent infection. Additionally IgM activity in neonates indicates congenital syphilis (3). Only ELISA, Immunoblot and 19S-IgM-FTA-ABS are able to differentiate between IgG and IgM antibodies.

The assessment of *Treponema pallidum*-specific IgM antibodies for the check of the treatment indication of a Treponema infection is suitable for the normal course of infection. A positive IgM antibody finding should, however, not be effected without the patient anamnesis (stage of infection, therapy), as the IgM antibodies, depending on the time interval between infection and start of the therapy, may remain detectable a few months up to several years (persistent IgM antibodies). At Neurosyphilis, Reactivation or Second Infection the IgM antibody synthesis may be nearly completely suppressed (4).

3. **Principle of Test**

Proteins of the pathogen-antigen (5,6) are transferred to the nitrocellulosis membrane by a micro-dispensing method. The nitrocellulosis membrane is then cut into single strips.

Incubation of the antigen-coated nitrocellulose strips with samples of human serum or plasma permits the detection of specific antibodies. These antibodies develop immunocomplexes with the antigen fixed on the test strip. After removing the unbound antibodies by washing steps, the single nitrocellulose-strips are incubated with alcalic phosphatasis conjugated anti-human IgG- respectively IgM-antibodies. After unbound conjugated antibodies have been removed by a further washing step, a visualisation of the antigen/antibody-complex (of the bound antibodies) is accomplished by the addition of a non-coloured substrate, which forms blue-violet precipitates at each site ("antigen bands") where the conjugated anti-human antibodies have bound. The enzyme/substrate-reaction is stopped through washing the nitrocellulose-strips with aqua dest./deionised. Depending on the observed band pattern one can interpret the presence of specific IgG- respectively IgM-antibodies.
4. Package Contents

4.1 Kit for 16 determinations

1. IgG resp. IgM Nitrocellulose test strips with sprayed antigen, (solid strips stabilised on a plastic foil), sorted in a booklet, ready to use 1x 16 strips
2. IgG resp. IgM Cut off Control, human serum, prediluted 1x 0,5ml
3. Dilution-/washbuffer, pH 7.3 (10x conc.), with Tris and preservative 1x 50 ml
4. IgG- resp. IgM- Conjugate (100x conc.)
   Anti-human-(goat)-Alcalic Phosphatasis, with preservative 1x 0,7 ml
5. Substrate (BCIP/NBT), ready to use 1x 57 ml
6. Evaluation Record sheet for the notation and storage of the results 1x 1 pcs.

4.2 Kit for 32 determinations

1. IgG resp. IgM Nitrocellulose test strips with sprayed antigen, (solid strips stabilised on a plastic foil), sorted in a booklet, ready to use 2x 16 strips
2. IgG resp. IgM Cut off Control, human serum, prediluted 1x 0,5ml
3. Dilution-/washbuffer, pH 7.3 (10x conc.), with Tris and preservative 2x 50 ml
4. IgG- resp. IgM- Conjugate (100x conc.)
   Anti-human-(goat)-Alcalic Phosphatasis, with preservative 1x 0,7 ml
5. Substrate (BCIP/NBT), ready to use 1x 57 ml
6. Evaluation Record sheet for the notation and storage of the results 1x 1 pcs.

Also available on request:
IgG or IgM- Positive control, human serum, prediluted, 0.5 ml.
The positive bands > For the cut--off band, refer to the certificate supplied with the kit.
(Order No.: IgG: WE150P60 or IgM: WE150P80)

IgG/IgM- Negative control, human serum, prediluted, 0.5 ml.
The negative control shows no bands or no bands relevant to the evaluation. > Cut-off band.
(Order No.: IgG/IgM: WE150N10)

5. Storage and Stability

Store test kit at 2-8°C. The shelf life of the single components is mentioned on the relevant label; for shelf life of the Kit please refer to the Quality Control Certificate.

1. Do not expose the single kit components to high temperature nor freeze them.
2. Do not use the kit reagents after their expiring date.
3. Do not expose reagents to strong light during storage or incubation.
4. The BCIP/NBT-substrate solution is sensitive to light and has to be stored in dark.
5. Nitrocellulose test strips : Use strips immediately after taken out of the bag. Close bag with the not required strips again savely and store at 2-8°C. When putting the results into archives please take care that the nitrocellulose test strips and templates are protected against direct sunlight, to avoid fading of the bands.

<table>
<thead>
<tr>
<th>Material</th>
<th>Status</th>
<th>Storage</th>
<th>Shelflife</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Samples</td>
<td>Undiluted</td>
<td>+2 to +8°C</td>
<td>1 week</td>
</tr>
<tr>
<td>Test Strips</td>
<td>After Opening</td>
<td>+2 to +8°C (stored in supplied bag)</td>
<td>3 months</td>
</tr>
</tbody>
</table>

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Treponema pallidum LINE IgG/IgM GB

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9. Pipette 1.5 ml of the prepared conjugate dilution each into the corresponding incubation channel and incubate for 30 minutes on the rocking platform.
10. Pour away or aspirate liquid completely out of the channels.
11. Washing of the strips: Incubate with 1.5 ml ready to use dilution-/washbuffer each for 3 x 5 minutes on the rocking platform. Pour away or aspirate the washbuffer always completely. Afterwards rinse 1 x 1 minute with Aqua dest./deionised.
12. Pour away or aspirate the liquid completely out of the channels (refer to point 6).
13. Pipette 1.5 ml ready to use substrate solution each into the channels and allow to develop 10 ± 3 minutes on the rocking platform.
14. Stop the color reaction by pouring away the substrate solution. Afterwards wash the strips without incubation in between for 3 x with 1.5 ml Aqua dest./deionised each.
15. Pour away the aqua dest./deionised and let the strip dry on a clean cellulosis paper. The background-coloring, that may be observed on the moistured antigen strips disappears completely when the strips are completely dry. Solid antigen strips need a little longer than the conventional antigen strips until they are completely dry.
16. Use the included calculation protocol for the interpretation. The inscription of the high-specific band on the protocol sheet make the interpretation of the patient samples easier for you.

For test procedure scheme pls. refer to last page

9.4 Use of Immunoblot-processors
The following instruments have been validated for the automatic processing of the Blots and LINEs: Apollo and Profiblot. All commercially available Blot machines are suitable in principle.

10. Interpretation of Results

For a secure interpretation each LINE is fitted out with two controls:
1. Serum control:
   Only after the incubation with patient serum the serum incubation band appears below the markline.
2. Conjugate control:
   The Treponema pallidum LINE strip is fitted out with an IgG- resp. IgM conjugate control band.

The test procedure is valid, if the serum control as well as the internal conjugate control appears clearly visible on the developed antigen strip.
Please refer to the protocol sheet for the information of the exact position of the serum- and the conjugate control.

10.1 Interpretation of the patient samples

Please refer to the protocol sheet for position and denotation of reactive bands.
IgG and IgM bands: TpN47, TmpA, TpN17, TpN15

10.2 Usage of the Cut-Off Control:

Bands with an intensity weaker than the cut-off band (TpN 47) of the cut-off control are not considered for the interpretation. The TpN47-band must show a weak intensity.

Assessment of the band intensities:

TpN 47-band: The intensity of the TpN47-band of the cut-off control is the basis of all protein bands in IgG and IgM and is defined as follows:

- Less intensity than the TpN 47-band of the cut-off control = 0
• Same intensity than the TpN 47-band of the cut-off control = 1
• Stronger intensity than the TpN 47-band of the cut-off control = 2

The sum of the band intensities makes the total assessment.

10.3 Meaning of the Antigens

Listing of the used recombinant proteins of the Treponema pallidum-antigen (5, 6).

<table>
<thead>
<tr>
<th>Antigen / Description</th>
<th>Meaning of the antigens</th>
<th>Specificity of the antibodies in the LINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TpN47</td>
<td>Marker for a primary, secondary and latent syphilis (5, 6)</td>
<td>Highspecific for all stages of infection</td>
</tr>
<tr>
<td>TmpA (TpN44,5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TpN17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TpN15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notice: The combination of the high-specific antigens mentioned in above table is based on the allegation of the patents (owner S. Krell) No.: DE 195 36 166 C1 and EP 0 855 032 B1, and the guidelines for the serological syphilis diagnostic, MIQ 2001: syphilis (Hagedorn) (4).

10.4 Interpretation criteria

The interpretation of the serological result shall always include the clinical picture, epidemiological data and further diagnostical parameter.

<table>
<thead>
<tr>
<th>IgG Assessment</th>
<th>IgM Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum of the band intensities</td>
<td>Sum of the band intensities</td>
</tr>
<tr>
<td>&lt; 3</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>= 3</td>
<td>= 2</td>
</tr>
<tr>
<td>&gt; 3</td>
<td>&gt; 2</td>
</tr>
<tr>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>suspicious (*)</td>
<td>suspicious (*)</td>
</tr>
<tr>
<td>positive</td>
<td>positive</td>
</tr>
</tbody>
</table>

(*) In case of a suspicious result, ask for a second serum sample and/or retest using a different test method.

10.5 Limits of the Test

1. A negative Blot result does not completely exclude the possibility of an infection with Treponema pallidum. The sample may be taken before the occurrence of antibodies, or the antibody titre exists below the detection limit of the test.
2. In rare cases patients may show „inverse“-bands (dark background, white bands), these are not to be considered, means the Immunoblot can not be assessed in such cases. The serum should be checked using other serological methods.
3. A diagnostic statement regarding Neurosyphilis and Neonate-Syphilis can not been made as corresponding sera respectively cerebrospinal fluid samples were not available for the evaluation.
4. Due to the high DNA-homology of T. pallidum subsp. pallidum (syphilis), endemicum (endemic syphilis) and pertenue (framboasis, yaws), partially also Treponema carateum (Pinta), cross-reactivities are to be expected. This means for the use of serological tests, a differential-diagnostical limitation of the non-venerial Treponematosis is impossible (4).
5. Lues latens patients sera may give in single cases discrepant results between 19S-IgM-FTA-ABS and recombinant Blot-tests as well as EIAs. The cause of this discrepancy is unclear so far.
6. For the interpretation of isolated borderline resp. positive IgM results of pregnant women the possibility of present multi-reactive IgM-antibodies has to be considered. Such results shall be further investigated with additional tests (19S-IgM-FTA-ABS (IgM-ELISA) or VDRL-test, pls. refer to “Diagnostic Meaning”).

11. Performance Data

11.1 Sensitivity IgG

In a study with sera material offered, beside others, by Prof. Dr. H.-J. Hagedorn, Herford, 298 IgG sera with suspected *Treponema pallidum* infection have been tested on the Virotech LINE IgG to determine the sensitivity for IgG. This patient material is composed of different sera-collectives (syphilis sera of primary- and secondary stage as well as Lues latens, routine sera, sera of prostitutes, a commercially available reference panel, sera of pregnant women, HIV-positive sera and follow-up sera).

The sera have been pre-determined with different reference-methods (Finding: Westernblot, TPHA, FTA, FTA-ABS, ELISA and VDRL).

<table>
<thead>
<tr>
<th>Sera Collective IgG (n=298)</th>
<th>LINE IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>Finding</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>36</td>
</tr>
<tr>
<td>Positive</td>
<td>12</td>
</tr>
</tbody>
</table>

One borderline result in IgG has not been considered for the sensitivity.

Referring to the finding (reference method) an sensitivity of 95,0% has been obtained for IgG

11.2 Sensitivity IgM

In a study of Prof. Dr. H.-J. Hagedorn, 135 IgM sera have been tested on the Virotech LINE IgM to determine the sensitivity for IgM. These sera have been pre-defined with the 19S-IgM-FTA-ABS as reference method (finding) and comprise syphilis-sera of the primary- and secondary stage as well as lues latens and others.

<table>
<thead>
<tr>
<th>Sera Collective IgM (n=135)</th>
<th>LINE IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>Finding</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>28</td>
</tr>
<tr>
<td>Positive</td>
<td>12</td>
</tr>
</tbody>
</table>

Borderline results in IgM have not been considered for the calculation of the sensitivity.

Referring to the finding (19S-IgM-FTA-ABS as reference method) an sensitivity for IgM of 87,4% has been obtained.

11.3 Specificity

For the determination of the specificity, a sera collective consisting of blood donor sera, potentially cross-reacting sera and pregnant women sera have been examined (IgG n=387 / IgM n=371)

<table>
<thead>
<tr>
<th>LINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
</tr>
<tr>
<td>383</td>
</tr>
<tr>
<td>3</td>
</tr>
</tbody>
</table>

Borderline results have not been considered for the calculation of the specificity.

The specificity is 99,2% for IgG and 98,6% for IgM
11.4 Diagnostic Sensitivity

The assessment of the diagnostic sensitivity is based on clinical defined data of the primary- and secondary stage (source of sera: Lab. Prof. Dr. J.-J. Hagedorn, Herford).

<table>
<thead>
<tr>
<th>Sera Collective IgG (n=32)</th>
<th>LINE IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>Diagnostic Finding/Clinic</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sera Collective IgM (n=33)</th>
<th>LINE IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>Diagnostic Finding/Clinic</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
</tr>
</tbody>
</table>

Borderline results have not been considered for the calculation of the diagnostic sensitivity.

The results in the above table show that all clinical defined sera are detected correctly in the total finding.

11.5 Cross-Reactivity

Cross-reactions with antibodies against partial antigens of the species of the family Spirochaetaceae are described in the literature (7). Both, the species Treponema as well as the species Borrelia belong to this family. However, crossreacting antibodies against the antigens TpN47, TmpA, Tp17 and Tp15 used for the Virotech LINE are not described. In-house tests of Borrelia positive sera showed a negative result for Treponema antibodies. Furthermore, sera of patients with Systemic Lupus Erythenatodes (SLE) have been tested.

The results were negative as well.

11.6 Prevalence (Expected values)

The following table shows the results of bloodbank-sera and pregnant women’s sera:

<table>
<thead>
<tr>
<th></th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative</td>
<td>222</td>
<td>188</td>
</tr>
<tr>
<td>borderline</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>positive</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

The positive tested serum is a blooddonor serum.

11.7 Intra-Assay-Precision (Repeatability)

For the determination of the repeatability, 32 blot strips of a non-cut Nitrocesillulose-membrane have been incubated in a first examination testrun with the cut-off control and in a second examination testrun with the positive control. The bands show an uniform intensity on the whole nitrocellulose-sheet.

11.8 Inter-Assay-Precision (Reproducibility)

The determination of the test precision has been effected in 10 independent test runs, both, manually and using automates and all proceeded by different persons.

A negative serum, a low positive serum and a positive serum have been tested in IgG and in IgM.
<table>
<thead>
<tr>
<th></th>
<th>IgG</th>
<th></th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>10</td>
<td>Negative</td>
<td>10</td>
</tr>
<tr>
<td>Low Positive</td>
<td>10</td>
<td>Low Positive</td>
<td>6/4 (*)</td>
</tr>
<tr>
<td>Positive</td>
<td>10</td>
<td>Positive</td>
<td>10</td>
</tr>
</tbody>
</table>

(*) The IgM low positive serum has been assessed 6x positive and 4x borderline in a total of 10 testruns.

12. References

13. Test Procedure Scheme

**Test Procedure in short version**

<table>
<thead>
<tr>
<th>Step</th>
<th>Time/Action</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples Incubation</td>
<td>30 minutes</td>
<td>15 µl Patient serum/ plasma/ 100 µl control in 1,5 ml dilution/-washbuffer each</td>
</tr>
<tr>
<td>Washing</td>
<td>3 x 5 minutes</td>
<td>with 1,5 ml dilution/-washbuffer each</td>
</tr>
<tr>
<td>Conjugate incubation</td>
<td>30 minutes</td>
<td>with 1,5 ml working dilution (1 + 100)</td>
</tr>
<tr>
<td>Washing</td>
<td>3 x 5 minutes</td>
<td>with 1,5 ml dilution/-washbuffer each</td>
</tr>
<tr>
<td></td>
<td>1 x 1 minute</td>
<td>with Aqua dest./deionised</td>
</tr>
<tr>
<td>Substrate incubation</td>
<td>10 ± 3 minutes</td>
<td>with 1,5 ml ready to use substrate solution each</td>
</tr>
<tr>
<td>Stopping</td>
<td>3 x without incubation in between</td>
<td>with 1,5 ml Aqua dest./deionised each</td>
</tr>
</tbody>
</table>

**Conjugate Dilution table (rounded)**

| Number of strips | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
|------------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Dilution/-washbuffer | 1,5ml | 3,0ml | 4,5ml | 6,0ml | 7,5ml | 9,0ml | 11,0ml | 12,0ml | 14,0ml | 15,0ml | 17,0ml | 18,0ml | 20,0ml | 21,0ml | 23,0ml | 24,0ml | 26,0ml | 27,0ml | 29,0ml | 30,0ml | 32,0ml | 33,0ml | 35,0ml | 36,0ml | 38,0ml | 39,0ml | 41,0ml | 42,0ml | 44,0ml | 45,0ml |
| Conjugate-concentrate  | 15µl | 30µl | 45µl | 60µl | 75µl | 90µl | 110µl | 120µl | 140µl | 150µl | 170µl | 180µl | 200µl | 210µl | 230µl | 240µl | 260µl | 270µl | 290µl | 300µl | 320µl | 330µl | 350µl | 360µl | 380µl | 390µl | 410µl | 420µl | 440µl | 450µl |
| Final volume           | 1,515m | 3,03ml | 4,545m | 6,06ml | 7,575m | 9,09ml | 11,11m | 12,12m | 14,14m | 15,15m | 17,17m | 18,18m | 20,2ml | 21,21m | 23,23m | 24,24m | 26,26m | 27,27m | 29,29m | 30,3ml | 32,32m | 33,33m | 35,35m | 36,36m | 38,38m | 39,39m | 41,41m | 42,42m | 44,44m | 45,45m |

| Number of strips | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 |
|------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Dilution/-washbuffer | 47,0ml | 48,0ml | 50,0ml | 51,0ml | 53,0ml | 54,0ml | 56,0ml | 57,0ml | 59,0ml | 60,0ml | 47,47m | 48,48m | 50,5ml | 51,51m | 53,53m | 54,54m | 56,56m | 57,57m | 59,59m | 60,6ml | 470µl | 480µl | 500µl | 510µl | 530µl | 540µl | 560µl | 570µl | 590µl | 600µl | 47,47m | 48,48m | 50,5ml | 51,51m | 53,53m | 54,54m | 56,56m | 57,57m | 59,59m | 60,6ml |