

## **VIROTECH Treponema pallidum IgG LINE Immunoblot**

**(T. pallidum IgG LINE-16)**

**Order No.: WE150G16**

**(T. pallidum IgG LINE-32)**

**Order No.: WE150G32**

## **VIROTECH Treponema pallidum IgM LINE Immunoblot**

**(T. pallidum IgM LINE-16)**

**Order No.: WE150M16**

**(T. pallidum IgM LINE-32)**

**Order No.: WE150M32**

**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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REV 22 / VIROTECH T. pallidum IgG & IgM LINE Immunoblot GB

# Contents

<b>1.</b>	<b>Intended Use.....</b>	<b>3</b>
<b>2.</b>	<b>Diagnostic Meaning.....</b>	<b>3</b>
<b>3.</b>	<b>Principle of Test.....</b>	<b>3</b>
<b>4.</b>	<b>Package Contents.....</b>	<b>4</b>
4.1	Kit for 16 determinations .....	4
4.2	Kit for 32 determinations .....	4
<b>5.</b>	<b>Storage and Stability .....</b>	<b>4</b>
<b>6.</b>	<b>Precautions and Warnings .....</b>	<b>5</b>
<b>7.</b>	<b>Additionally required material (not supplied).....</b>	<b>5</b>
<b>8.</b>	<b>Examination Material .....</b>	<b>5</b>
<b>9.</b>	<b>Test Procedure .....</b>	<b>5</b>
9.1	Preparation of Samples.....	5
9.2	Preparation of Reagents .....	6
9.3	Immunoblot Test Procedure.....	6
9.4	Use of Immunoblot-processors .....	7
<b>10.</b>	<b>Interpretation of Results.....</b>	<b>7</b>
10.1	Interpretation of the patient samples .....	7
10.2	Usage of the Cut-Off Control: .....	7
10.3	Meaning of the Antigens .....	8
10.4	Interpretation criteria .....	8
10.5	Limits of the Test .....	8
<b>11.</b>	<b>Performance Data .....</b>	<b>9</b>
11.1	Sensitivity IgG.....	9
11.2	Sensitivity IgM .....	9
11.3	Specificity.....	9
11.4	Diagnostic Sensitivity .....	10
11.5	Cross-Reactivity .....	10
11.6	Prevalence (Expected values) .....	10
11.7	Intra-Assay-Precision (Repeatability) .....	10
11.8	Inter-Assay-Precision (Reproducibility) .....	10
<b>12.</b>	<b>References.....</b>	<b>11</b>
<b>13.</b>	<b>Test Procedure Scheme .....</b>	<b>12</b>

## 1. Intended Use

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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

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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

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Proteins of the pathogen-antigen (5,6) are transferred to the nitro cellulose membrane by a micro-dispensing method. The nitro cellulose 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 immune complexes with the antigen fixed on the test strip. After removing the unbound antibodies by washing steps, the single nitrocellulose-strips are incubated with alkaline phosphatase 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 distilled or deionized water. 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. <b>IgG resp. IgM Nitrocellulose test strips</b> with sprayed antigen, (solid strips stabilised on a plastic foil), sorted in a booklet, ready to use | <b>1x</b> | 16 strips |
| 2. <b>IgG resp. IgM Cut off Control, human serum, prediluted</b>  | <b>1x</b> | 1.0ml     |
| 3. <b>Dilution-/ washbuffer</b> , pH 7.3 (10x conc.), with Tris and preservative  | <b>1x</b> | 50 ml     |
| 4. <b>IgG- resp. IgM- Conjugate</b> (100x conc.)<br><b>Anti-human-(goat)-Alkaline Phosphatase</b> , with preservative                                   | <b>1x</b> | 0.7 ml    |
| 5. <b>Substrate</b> (BCIP/NBT), ready to use  | <b>1x</b> | 57 ml     |
| 6. <b>Evaluation Record sheet</b> for the notation and storage of the results   | <b>1x</b> | 1 pcs.    |

### 4.2 Kit for 32 determinations

- |   |           |           |
|---|-----------|-----------|
| 1. <b>IgG resp. IgM Nitrocellulose test strips</b> with sprayed antigen, (solid strips stabilised on a plastic foil), sorted in a booklet, ready to use | <b>2x</b> | 16 strips |
| 2. <b>IgG resp. IgM Cut off Control, human serum, prediluted</b>  | <b>1x</b> | 1.0ml     |
| 3. <b>Dilution-/ washbuffer</b> , pH 7.3 (10x conc.), with Tris and preservative  | <b>2x</b> | 50 ml     |
| 4. <b>IgG- resp. IgM- Conjugate</b> (100x conc.)<br><b>Anti-human-(goat)- Alkaline Phosphatase</b> , with preservative                                  | <b>1x</b> | 0.7 ml    |
| 5. <b>Substrate</b> (BCIP/NBT), ready to use  | <b>1x</b> | 57 ml     |
| 6. <b>Evaluation Record sheet</b> for the notation and storage of the results   | <b>1x</b> | 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 safely 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.

Material	Status	Storage	Shelf life
Test Samples	Undiluted	+2 to +8°C	1 week
Test Strips	After Opening	+2 to +8°C (stored in supplied bag)	3 months

Controls	After Opening	+2 to +8°C	3 months
Conjugate	After Opening	+2 to +8°C	3 months
	Diluted	+2 to +8°C	ca. 6h
Substrate	After Opening	+2 to +8°C (protect from light)	3 months
Washing Solution	After Opening	+2 to +8°C (protect from light)	3 months
	Final Dilution (ready-to-use)	+2 to +8°C	4 weeks
	Final Dilution (ready-to-use)	or room temperature	2 weeks

## 6. Precautions and Warnings

1. Only sera, that have been tested and found to be negative for HIV1-ab, HIV2-ab, HCV-ab and Hepatitis-B-surface-antigen are used as control sera. Nevertheless, samples, diluted samples, controls and conjugate as well as the antigen strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.
2. Use plastic forceps and wear protective gloves when handling the Immunoblot.
3. Please follow the local valid waste disposal regulations.
4. The incubation baths are designed by the manufacturer for a single use. The reuse of the incubation baths is at the risk of the user. If they are to be reused we recommend that after use the incubation baths be disinfected for several hours in 1% sodium hypochlorite solution and then rinsed thoroughly with tap water followed by distilled or deionized water

## 7. Additionally required material (not supplied)

1. Incubation tray (if required available with order no.: WE300.08)
2. Rocking platform (vertical not centrifugal)
3. A wash bottle for stopping
4. Pipette or handwasher
5. Micro-pipettes 5 µl - 1500 µl
6. Pipette filler
7. Test tubes, 2-20 ml volume
8. Plastic forceps
9. Distilled or deionized water
10. Filter paper

## 8. Examination Material

Either serum and plasma may be used as test materials, even when the package leaflet only mentions serum. Plasma samples may contain any anticoagulant.

## 9. Test Procedure

Working exactly referring to the VIROTECH Diagnostics user manual is the prerequisite for obtaining correct results.

### 9.1 Preparation of Samples

1. 15 µl serum or plasma are needed for each patient sample.
2. Blood samples should be taken separately by venous puncture. Serum is separated after complete coagulation (not applicable to plasma). If they are to be stored longer sera have to be frozen at -20°C.
3. Repeated freezing and thawing should be avoided.
4. Sera that are heat-inactivated, lipemic, haemolytic or microbiologically contaminated, may lead to faulty results and shall therefore not be used.

5. Do not use turbid samples (especially after thawing), centrifuge if necessary (5 minutes at 1000sg), pipette clear supernatant and use in testing.

## 9.2 Preparation of Reagents

1. To facilitate routine laboratory work, all LINEs and EcoBlots can be processed in a single test run with the same incubation times and the same component - when these are independent of the parameters and batches. The cut off controls now have parameter and batch specific values.
2. Bring the corresponding concentrate to room temperature (20-25°C) before preparing the dilution. Use only high quality distilled or deionized water and bring up to room temperature (20-25°C) before usage.
3. Mix dilutions well before starting the test.
4. **Dilution-/Washbuffer:**  
The dilution-/washbuffer is provided as a 10-fold concentrate. Dilute the dilution-/washbuffer concentrate 1:10 with distilled or deionised water (10ml/50ml/100ml concentrate + 90ml/450ml/900ml A distilled or deionised water), mix well. Both the concentrated and the diluted dilution/washing buffer may exhibit a yellow colouration. This colouration does not influence the stability of the dilution/washing buffer or the function or the reliability of the diagnostic test.
5. **IgG resp. IgM conjugate**  
Dilute the conjugate 1 + 100 with finally diluted dilution/washing buffer and mix thoroughly. 1.5 ml conjugate working solution is required for each serum sample. See conjugated dilution table (item: "Test Procedure").
6. **Substrate Solution**  
The substrate solution is delivered ready-to-use.

## 9.3 Immunoblot Test Procedure

**Attention:** The antigenstrips must only be tested in the released Ig-class.  
(pls. refer to the label on the blot booklet and the marking on each single test strip).

For the correct performance and evaluation of the *Treponema pallidum* LINEs, each test run should include the appropriate parameter and batch-specific cut off controls.

<p>For a secure <i>Treponema pallidum</i> diagnostic the LINE shall be proceeded in IgG and IgM.</p>
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1. Test has to be proceeded at room temperature.
2. For each sample put 1 strip into the channel of a clean incubation tray. Hold strip only at the marked upper end.
3. Pipette 1,5ml ready to use **dilution-/ washbuffer** each and put onto the rocking platform. Take care that the antigen strips are consistently covered with liquid, the strips must not dry out during the whole test procedure.
4. The solid antigen strips are being moisturized completely within one minute and can be incubated in supine, lateral position or face-down position.
5. **15 µl patient serum or plasma or 100 µl of the cut-off or positive / negative control** added by pipetting, if at all possible at the upper marked end of the strip. Incubate patient serum and control for **30 minutes** on the rocking platform. Take care that during pipetting and following pour away no cross-contamination of the single patient samples occur.
6. Aspirate or carefully pour away the liquid out of the channels completely. During the pour away of the liquid, the antigen strips remain at the bottom of the channel. Drain the remaining liquid onto a cellulose paper.
7. **Washing** of 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 washing buffer always completely. Before ending of the last washing step, prepare the needed amount of fresh conjugate dilution (refer to table).
8. Aspirate or pour away the liquid completely out of the channels (please refer to point 6).
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 **distilled or deionized water**.
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 colour reaction by pouring away the substrate solution. Afterwards wash the strips without incubation in between for **3 x** with 1,5 ml **distilled or deionized water** each.
15. Pour away the distilled or deionized water and let the strip dry on a clean cellulose paper. The background-colouring, that may be observed on the moisturized 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
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#### 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

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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 mark line.
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).

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

**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.

IgG Assessment	
Sum of the band intensities	Interpretation
< 3	negative
= 3	suspicious (*)
> 3	positive

IgM Assessment	
Sum of the band intensities	Interpretation
< 2	negative
= 2	suspicious (*)
> 2	positive

(\*) 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 could not have been made as corresponding 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 Treponematoses 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).

Sera Collective IgG (n=298)		LINE IgG	
		Negative	Positive
Finding	Negative	36	7
	Positive	12	230

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

**Referring to the finding (reference method) on 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.

Sera Collective IgM (n=135)		LINE IgM	
		Negative	Positive
Finding	Negative	28	0
	Positive	12	83

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) on 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)

	LINE	
	IgG	IgM
negative	383	356
positive	3	5

**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).

Sera Collective IgG (n=32)		LINE IgG	
		Negative	Positive
Diagnostic Finding/Clinic	Negative	-	-
	Positive	-	32

Sera Collective IgM (n=33)		LINE IgM	
		Negative	Positive
Diagnostic Finding/Clinic	Negative	1	0
	Positive	2	26

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, cross reacting 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 Erythematosus (SLE) have been tested.

The results were negative as well.

#### 11.6 Prevalence (Expected values)

The following table shows the results of blood bank-sera and pregnant women's sera:

	IgG	IgM
negative	222	188
borderline	-	5
positive	1	-

The positive tested serum is a serum of a blood donor.

#### 11.7 Intra-Assay-Precision (Repeatability)

For the determination of the repeatability, 32 blot strips of a non-cut Nitrocellulose-membrane have been incubated in a first examination run with the cut-off control and in a second examination run 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.

	<b>IgG</b>
<b>Negative</b>	10
<b>Low Positive</b>	10
<b>Positive</b>	10

	<b>IgM</b>
<b>Negative</b>	10
<b>Low Positive</b>	6/4 (*)
<b>Positive</b>	10

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

## 12. References

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### 13. Test Procedure Scheme

#### Test Procedure in short version

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

#### Conjugate Dilution table (rounded)

Number of strips	1	2	3	4	5	6	7	8	9	10
Dilution-/washbuffer	1,5ml	3,0ml	4,5ml	6,0ml	7,5ml	9,0ml	11,0ml	12,0ml	14,0ml	15,0ml
Conjugate-concentrate	15µl	30µl	45µl	60µl	75µl	90µl	110µl	120µl	140µl	150µl
Final volume	1,515m	3,03ml	4,545m	6,06ml	7,575m	9,09ml	11,11m	12,12m	14,14m	15,15m

Number of strips	11	12	13	14	15	16	17	18	19	20
Dilution-/washbuffer	17,0ml	18,0ml	20,0ml	21,0ml	23,0ml	24,0ml	26,0ml	27,0ml	29,0ml	30,0ml
Conjugate-concentrate	170µl	180µl	200µl	210µl	230µl	240µl	260µl	270µl	290µl	300µl
Final volume	17,17m	18,18m	20,2ml	21,21m	23,23m	24,24m	26,26m	27,27m	29,29m	30,3ml

Number of strips	21	22	23	24	25	26	27	28	29	30
Dilution-/washbuffer	32,0ml	33,0ml	35,0ml	36,0ml	38,0ml	39,0ml	41,0ml	42,0ml	44,0ml	45,0ml
Conjugate-concentrate	320µl	330µl	350µl	360µl	380µl	390µl	410µl	420µl	440µl	450µl
Final volume	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
Dilution-/washbuffer	47,0ml	48,0ml	50,0ml	51,0ml	53,0ml	54,0ml	56,0ml	57,0ml	59,0ml	60,0ml
Conjugate-concentrate	470µl	480µl	500µl	510µl	530µl	540µl	560µl	570µl	590µl	600µl
Final volume	47,47m	48,48m	50,5ml	51,51m	53,53m	54,54m	56,56m	57,57m	59,59m	60,6ml