recomLine Treponema IgG, IgM

**1 Purpose**
recomLine Treponema IgG, IgM is an immunocassay for the qualitative determination of IgG and IgM antibodies to Treponema pallidum in human serum or plasma.

**2 Intended use**
The recomLine Treponema IgG, IgM can be used as a confirmatory test in the screening of reactive samples. The recomLine Treponema IgG, IgM is a line immunocassay. In contrast to ELISAs, this testing principle allows the separate lining up of individual antigens and thus the determination of specific antibodies to individual Treponema pallidum antigens. The test uses recombinantly-produced antigens: Tp47, TmpA, Tp257 (Gpd), Tp453, Tp17 and Tp15.

**3 Test principle**
Highly-purified, recombinant Treponema antigens are fixed on nitrocellulose membrane strips.

1. The test strips are incubated with the diluted serum or plasma sample, and the specific antibodies bind to the pathogen antigens on the test strips.
2. Unbound antibodies are then flushed away.
3. In a second step, the strips are incubated with anti-human immuno- globulin antibodies (IgG and /or IgM), which are coupled to horse-radish peroxidase.
4. Unbound conjugate antibodies are then flushed away.
5. Specifically bound antibodies are detected with a staining reaction catalysed by the peroxidase. If an antigen-antibody reaction has taken place, a dark band will appear on the strip at the corresponding point.

There are control bands at the upper end of the test strips:
- **a**): The reaction control is located under the strip number, and must be present for the test to be valid.
- **b**): The reaction is catalysed by the peroxidase. The reaction control will show a strong reaction; while the IgM class specific. If the IgG class specific is used, the same reagents (see printed symbol) can be used across the whole range of parameters and batches. The shelf life of these components should be noted.
- **c)**: "Cut-off control": The intensity of this band allows the assessment of the reactivity of each antigen band (see 9.2. Evaluation).

**4 Reagents**

**4.1 Package contents**
The reagents in one package are sufficient for 20 (100) tests.

Each test kit contains:

- **WASHBUF A (10X)** 100 ml (5x100 ml) Wash Buffer A (10 times concentration)
- **SUSBS** 40 ml (10x40 ml) Chromogenic Substrate Tetramethylbenzidine (TMB, ready-to-use)
- **MILKPOW** 5 g (5x5 g) skimmed milk powder
- **INSTRU** 1 Instructions for use
- **EVALFORM** 1 (b) Evaluation form

**4.1.1 recomLine Treponema IgG**
In addition to the components listed in 4.1, each test kit contains:

| TESTSTR | 2 (10) tubes, each with 10 numbered test strips |
| CONJ IgG | 500 µl (5x500 µl) anti-human lgG conjugate (100-fold concentration, green cap) From rabbit, contains NaN3 (<0.1%), MIT (<0.1%) and chlorazetamide (<0.1%) |

**4.1.2 recomLine Treponema IgM**
In addition to the components listed in 4.1, each test kit contains:

| TESTSTR | 2 (10) tubes, each with 10 numbered test strips |
| CONJ IgM | 500 µl (5x500 µl) anti-human lgM conjugate (hundred times concentrated, purple screw cap) From rabbit, contains NaN3 (<0.1%), MIT (<0.1%) and chlorazetamide (<0.1%) |

**4.2 Additional reagents, materials and devices required**
- Incubation trays (can be purchased as needed from MIKROGEN)
- Deionised water (high quality)
- Plastic forceps
- Horizontal shaker
- Vortex mixer or other rotators
- Vacuum pump or similar device
- Volumetric cylinders, 50 ml and 1000 ml
- Micropipettes with disposable tips, 20 µl and 1000 µl
- 10 ml pipette or dispenser
- Timer
- Absorbent paper towels
- Disposable protective gloves
- Waste container for bio-hazardous materials

**5 Shelf life and handling**
- Store reagents at +2°C to +8°C before and after use, do not freeze.
- Subject all ingredients for at least 30 minutes to room temperature (+18°C to +25°C) before beginning the test. The test procedure is carried out at room temperature.
- Where different recomLine and recomBlot tests are used, the same reagents (see printed symbol) can be used across the whole range of parameters and batches. The shelf life of these components should be noted.
- Mix the concentrated reagents and samples thoroughly before use.
- Avoid a build up of foam.
- Only open the tube containing the test strip immediately before use to avoid condensation. Leave unused strips in the tube and continue to store at +2°C to +8°C (resell tube tightly, test strips may not become moist before the test!).
- The strips are marked with the serial number, as well as the test code.
- The packages bear an expiration date. After this has been reached no guarantee of quality can be offered.
- Protect kit components from direct sunlight throughout the entire test procedure. The substrate solution (TMB) is especially sensitive to light.
- The test should only be carried out by trained and authorised personnel.
- In case of substantial changes to the product or the regulations for use by the user, the application may lie outside the purpose given by MIKROGEN.
- Cross-contamination of patient samples or conjugates can lead to inaccurate test results. Add patient samples, test strips and conjugate solution carefully. Make sure that incubation solutions do not flow over into other wells. Carefully remove liquids.
- The strips must be completely wetted and submerged throughout the entire procedure.
- Automation is possible; further information can be obtained from MIKROGEN.

**6 Warnings and precautions**
- For in vitro diagnostic use only
- All blood products must be treated as potentially infectious.
- The test strips were prepared with inactivated bacterial or viral antigens.
- After the addition of patient or control specimens the strip material must be considered infectious and treated as such.
- Suitable disposable gloves must be worn throughout the entire test procedure.
- The reagents contain the antimicrobial agents and preservatives sodium azide, MIT (methylisothiazolone), oxyquinol and chloracetamide and hydrogen peroxide. Avoid contact with the skin or mucous membrane. Sodium azide can form an explosive azide upon contact with heavy metals such as copper and lead azide.
- All pipetted liquids must be collected. All collecting containers must contain suitable disinfectants for inactivation of human pathogens. All reagents and materials contaminated with potentially infectious samples must be treated with disinfectants or disposed of according to your hygiene regulations. The concentrations and incubation periods of the manufacturer must be observed.
- Use incubation trays only once.
- Handle strips carefully using plastic forceps.
7 Sampling and preparation of reagents

7.1 Samples

The sample can be serum or plasma (citrate, EDTA, heparin, CPD), which needs to be separated from the blood clot as soon as possible after sampling so as to avoid haemolysis. Avoid Microbial contamination of the samples. Insoluble substances must be removed from the sample before incubation.

The use of heat-inactivated, icteric, haemolytic, lipemic or turbid samples is not recommended.

Caution!

If tests are not carried out immediately, samples can be stored for up to 2 weeks at +2 to +8°C. More extended storage of the samples is possible at -20°C or lower. Repeated freezing and thawing of samples is not recommended due to the risk of inaccurate results.

7.2 Preparation of solutions

7.2.1 Preparation of ready-to-use wash buffer A

This buffer is required for sample and conjugate dilutions, as well as for the washing stages.

The volume of wash buffer A for the corresponding number of tests must be determined according to the following formula (device-specific dead volume is not considered):

\[ \text{Wash buffer A concentrate [ml]} = \text{number of strips} \times 10 + \text{specific dead volume} \]

The quantities required for a defined number of test strips are to be mathematically determined according to the following formula:

\[ \text{Wash buffer A concentrate [ml]} = \text{number of strips} \times 0.1 \]

7.2.2 Preparation of conjugate solutions

The conjugate solution must be prepared just before use. It is not possible to store the ready-to-use conjugate solution.

One part of the conjugate concentrate is diluted with 100 parts of ready-to-use wash buffer A (1+100).

The quantities required for a defined number of test strips are to be calculated according to the following formula:

\[ \text{Conjugate concentrate [µl]} = \text{number of strips} \times 2 \]

7.3 Preparation of test strips

Prepare test strips before beginning the test. Do not touch the strips with bare hands. Use forceps to handle them.

Prepare strips for 2 ml of ready-to-use wash buffer A.

Important:

IgG and IgM strips are not interchangeable!

8 Test procedure

8.1 One hour of serum incubation

No. Execution Note
1 Subject all reagents for at least 30 minutes at 18°C - 25°C (room temperature) before beginning the test.

The test procedure is carried out at room temperature.

2 Prepare test strips

Place the strips in 2 ml of ready-to-use wash buffer A.

Do not touch the strips with bare hands - use the forceps. The strip number points upward.

Place each strip in a separate well in the incubation tray (see 4.2). The strips must be completely immersed.

3 Incubation of samples

a) 20 µl of undiluted sample (human serum or plasma) is pipetted on to the test strip for each incubation mixture. (Dilution 1 + 100)

Pipette the sample at one end of the immersed strip in the wash buffer A and mix as quickly as possible by carefully shaking the tray.

Cover the incubation tray with a plastic cover and place in the shaker.

b) Incubate for 1 hour with gentle shaking

4 Washing

a) Carefully remove the plastic cover from the incubation trays.

b) Gently siphon serum dilution from the individual wells.

c) Pipette 2 ml ready for use wash buffer A in every well, wash for 5 minutes with gentle shaking and then siphon off the wash buffer A.

5 Incubation with conjugate

Add 2 ml ready-to-use conjugate solution and incubate for 45 minutes with gentle shaking.

Cover the incubation tray with plastic cover and place in the shaker.

6 Washing

Carry out the washing stages three times in all (see 8.4a-8.4c).

7 Substrate reaction

Add 1.5 ml ready-to-use substrate solution and incubate for 8 minutes with gentle shaking.

8 Stopping the reaction

Remove substrate solution. Wash at least three times briefly with deionised water.

9 Drying the strips

Dry strip between 2 layers of absorbent paper for 2 hours before analysis.

Carefully remove strips from wafer using plastic forceps. Store strip away from light.

Caution!

Incubation solutions must not flow into other wells. Splashing must be avoided especially when opening and closing the lid.

8.2 Three hours of serum incubation

Alternatively, the test can be performed with three hours of serum incubation, with the only difference to the procedure described under 8.1. being points 3a) and 3b).

3 Incubation of samples

a) 20 µl of undiluted sample (human serum or plasma) is pipetted on to the test strip for each incubation mixture. (Dilution 1 + 200)

Pipette the sample at one end of the immersed strip in the wash buffer A and mix as quickly as possible by carefully shaking the tray.

Cover the incubation tray with a plastic cover and place in the shaker.

b) Incubate for 3 hours with gentle shaking.

9 Results

Caution:

Please do not use automated interpretation without consideration of the information on interpretation given below.

9.1 Validation - Quality Control

An analysis of the test can be carried out if the following criteria have been fulfilled:

1. Reaction control band (uppermost line) is clearly stained, dark band

2. Antibody class (second band): the IgG and/or IgM conjugate control band must show clear staining.

3. Cut-off control (third band): weak, but visible staining

9.2 Evaluation

The analysis of the test strips can be visual or computer-assisted - using the test strip analysis accessory recom scan. The recom scan software is designed to support the evaluation of test strips. Further information and related instructions for the computer-assisted analysis is available on request from MIKROGEN. The following instructions relate to visual analysis.

9.2.1 Assessment of band intensity

1. Note the date and batch number, as well as the detected antibody class, on the attached evaluation form.

2. Enter the sample identification numbers in the evaluation sheet.

3. Now stick the corresponding test strip onto the appropriate fields on the evaluation form using a glue stick. Align the test strip with the reaction control bands along the marked lines. Then use a transparent adhesive tape to attach the test strip to the left of the marked lines (do not tape over the reaction control band). Sticking the entire test strip down flat using glue or tape can lead to changes in colour.

4. Now identify the bands of the developed test strip on the basis of the printed control strip of the evaluation sheet and enter this in the evaluation sheet. For this purpose, carry out the assessment of the intensity of the occurring bands on the basis of Table 1 separately for the corresponding immunoglobulin classes.
Table 1: Assessment of band intensity in relation to the cut-off band

<table>
<thead>
<tr>
<th>Stain intensity of the bands</th>
<th>Assessment</th>
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</thead>
<tbody>
<tr>
<td>No reaction</td>
<td>-</td>
</tr>
<tr>
<td>Very low intensity (lower than weakly cutoff band)</td>
<td>+/-</td>
</tr>
<tr>
<td>Low intensity (equivalent to cutoff band)</td>
<td>+</td>
</tr>
<tr>
<td>Strong intensity (stronger than cutoff band)</td>
<td>++</td>
</tr>
<tr>
<td>Very strong intensity</td>
<td>+++</td>
</tr>
</tbody>
</table>

9.3 Interpretation of test results

Please see Table 2 for the test interpretation criteria.

Table 2: Test interpretation

<table>
<thead>
<tr>
<th>Test result</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>no antigen ≥ cut-off</td>
</tr>
<tr>
<td>Borderline</td>
<td>only one random antigen ≥ cut-off</td>
</tr>
<tr>
<td>Positive</td>
<td>at least two random antigens ≥ cut-off</td>
</tr>
</tbody>
</table>

10 Limitations of the method - restrictions

- Serological test results must always be considered in the context of other medical assessments of the patient. Therapeutic consequences of the serological findings must always be taken in context with the clinical data.
- Discussing possible cross-reactions is important for the interpretation of the test results. Like the genus Borrelia, the genus Treponema is part of the Spirochaetaeae family. The literature describes cross-reacting antibodies to partial antigens that are common to the Spirochaetaeae family (4).
- Cross-reacting antibodies to antigens TP47, TmpA, Tp257 (Gpd), Tp453, TP17 and TP15 as used in recomLine Treponema have not been described. They are characteristic Treponema pallidum antigens that show no reactivity to Borrelia-positive sera.
- A negative test result for recomLine Treponema cannot exclude an infection with Treponema pallidum. Further sampling and testing should be performed after four weeks with existing, clinical suspicion of infection with Treponema pallidum and negative, serological results.
- Positive IgG and/or IgM results are not always an indication for an active disease process.
- Dark test strips: Some patient samples can produce a dark, uniform or patterned staining across the entire nitrocellulose strip. Various factors in each patient serum are responsible for this. The evaluation of these strips is usually only partly feasible. Thus, "inverse" bands (white bands on dark background), for example, should be evaluated as negative. The respective serum should always be examined using other serological methods.

11 Test performance

11.1 Diagnostic sensitivity

<table>
<thead>
<tr>
<th>recomLine Treponema</th>
<th>Earlier positive findings in two reference tests</th>
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<tbody>
<tr>
<td></td>
<td>1 hour processing</td>
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<tr>
<td></td>
<td>IgG (n=280)</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td>Borderline</td>
<td>2</td>
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<tr>
<td>Positive</td>
<td>278</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100%*</td>
</tr>
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</table>

* Including inconclusive results.

11.2 Diagnostic specificity

<table>
<thead>
<tr>
<th>recomLine Treponema</th>
<th>Blood donor</th>
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<tr>
<td></td>
<td>1 hour processing</td>
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<tr>
<td></td>
<td>IgG (n=200)</td>
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<tr>
<td>Negative</td>
<td>199</td>
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<tr>
<td>Borderline</td>
<td>1</td>
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<td>Positive</td>
<td>2</td>
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<tr>
<td>Specificity</td>
<td>99.5%</td>
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</tbody>
</table>

11.3 Analytical specificity

The analytical specificity is defined as the capacity of the test to determine the analytes exactly in the presence of potential interference factors in the sample matrix or cross reactions with potentially interfering antibodies.

12 Literature

5. Sambri V., et al, Western Immunodotting with Five Treponema pallidum Recombinant Antigens for Serologic Diagnosis of Syphilis, Clinical and Diagnostic Laboratory Immunology, 2001 (8), Nr. 3: 534-539

Further information on Treponema diagnostics is available on request.

13 Explanation of symbols

<table>
<thead>
<tr>
<th>EVALFORM</th>
<th>CONTENTS</th>
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<tr>
<td>SUM</td>
<td>Evaluation form</td>
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<td>Instructions for use</td>
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<td>Contents, includes</td>
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14 Manufacturer and version information

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<th>recomLine Treponema IgM</th>
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<td>5172 (5173)</td>
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<td>Instructions for use</td>
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<td>valid from</td>
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GARLTP003EN_2013-04
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