*recom*Line Toxoplasma IgG [Avidität]
*recom*Line Toxoplasma IgM [IgA]

Instructions for use (English)

1 Purpose

The *recom*Line Toxoplasma IgG [Avidität], IgM [IgA] is a qualitative in-vitro test for the detection of IgG, IgM or IgA antibodies and the determination of the avidity of IgG antibodies against Toxoplasma gondii in human serum or plasma.

2 Intended use

The *recom*Line Toxoplasma makes use of phase-specific, recombinantly produced antigens and - unlike the lysate ELISA - it makes the safe identification of antibodies occurring in various phases of the infection process possible.
The *recom*Line Toxoplasma IgG [Avidität] can also be used to determine the IgG avidity. By combining the IgG band pattern with the determination of the avidity of phase-specific antibodies, the time of infection can be categorised under one of 3 different infection phases: acute infection, active infection (recent infection) and prior infection. By combining IgG avidity with proof of IgM, it can be clarified whether these are diagnostically relevant or persistent IgM antibodies.
To confirm the existing IgA results (e.g. ELISA), the IgA determination can take place with the aid of the *recom*Line Toxoplasma IgM [IgA] test. A phase-specific allocation of the IgA band pattern is not possible.

3 Test principle

Highly purified, recombinant toxoplasm antigens (ROP1c, MIC3, GRA7, GRA8, p30, MAG1, GRA1, rSAG1) are fixed on nitro-cellulose membrane test strips.

1. The test strips are incubated with the diluted serum or plasma sample, with specific antibodies attached to the pathogen antigens on the test strip.
2. Unbound antibodies are then flushed away.
3. In a second step, the strips are incubated with anti-human immunoglobulin antibodies (IgG, IgM and /or IgA), which are coupled to horseradish peroxidase.
4. Unbound conjugate antibodies are then flushed away.
5. Specifically bound antibodies are detected by a peroxidase-catalysed colour reaction. 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 band under the strip number, which must show a reaction for every serum/plasma sample.
b) The conjugate controls (IgG, IgM, IgA) serve as a control for the conjugate and strip type used (Ig class-specific). Where the IgG-specific test strip is used to detect IgG antibodies, the IgG conjugate control band shows a clear reaction; in the IgM- or IgA-specific test, the IgM or IgA control band must show a positive response.
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 tests.

Each test kit contains:

WASHBUF A 10 X	**100 ml** Wash Buffer A (**10 times concentration**) Contains phosphate buffer, NaCl, KCl, detergent, preservative: MIT (0.1%) and Oxypyrion (0.2%)
SUBS TMB	**40 ml** Chromogenic substrate Tetramethylbenzidin (**TMB, ready-to-use**)
MILKPOW	**5 g** skimmed milk powder
INSTRU	**1** Instructions for use
EVALFORM	**1** Evaluation form

4.1.1 *recomLine Toxoplasma IgG [Avidität]*

In addition to the components listed in 4.1, each test kit contains:

TESTSTR	**2** tubes, each with 10 numbered test strips
CONJ IgG	**500 µl** anti-human IgG conjugate **(100-fold concentration, green cap)** From rabbit, containing NaN3 (<0.1%), MIT (0.1%) and chloroacetamide (0.1%)

4.1.2 *Determining the avidity*

For the determination of the avidity of toxoplasm IgG antibodies, the avidity reagent, accompanied by the corresponding user instructions, may be provided as an additional product.

AVIDI Item No. 11010	**1** avidity reagent (solid 25g) for 60 ml of ready-to-use solution

4.1.3 *recomLine Toxoplasma IgM [IgA]*

In addition to the components listed in 4.1, each test kit contains:

TESTSTR	**2** tubes, each with 10 numbered test strips
CONJ IgM	**500 µl** anti-human IgM conjugate **(100-fold concentration, purple cap)** From rabbit, contains NaN_3 (<0.1%), MIT (<0.1%) and chlorazetamide (<0.1%)

4.1.4 *IgA determination*

Also available for the determination of IgA antibodies is *recom*Line Toxoplasma IgM kit IgA conjugate:

CONJ IgA Art. No. 10016	**500 µl** anti-human IgA conjugate **(100-fold concentration, colourless cap)** From rabbit, containing NaN3 (<0.1%), MIT (0.1%) and chloroacetamide (0.1%)

4.2 Materials required but not supplied

- 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 to +8 °C before and after use, **do not freeze**.
- Subject all ingredients to room temperature (+18 to +25 °C) for at least 30 minutes before beginning the test. The test procedure is carried out at room temperature.
- Washing Buffer, Milk Powder, Dilution Buffer, Conjugate and TMB can be interchanged between the different recomLine and recomBlot test systems, if these components carry the same symbols. Consider the shelf life of these components.
- Mix the concentrated reagents and samples thoroughly before use. Avoid the 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 (reseal tube tightly, test strips must 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 significant changes by the user to the product and/or the instructions for use, application may be beyond the purpose specified 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 immersed throughout the entire procedure.
- Automation is possible; you will receive further information 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 manufactured with inactivated whole cell lysates and / or recombinant produced bacterial, viral or parasitic 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), oxypyrion and chloroacetamide 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 siphoned liquids must be collected. All collecting containers must contain suitable disinfectants for the 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 stated by the manufacturer must be observed.
- Use incubation trays only once.
- Handle strips carefully using plastic forceps.
- Do not substitute or mix the reagents with reagents from other manufacturers.
- Read through the entire instructions for use before carrying out the test and follow them carefully. Deviation from the test protocol provided in the instructions for use can lead to erroneous results.

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 prior to incubation.
The use of heat-inactivated, icteric, haemolytic, lipemic or turbid samples is not recommended.
Caution!
If the tests are not conducted immediately, the sample 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 producing inaccurate results. Avoid more than 3 cycles of freezing and thawing

7.2 Preparation of solutions

7.2.1 *Preparation of ready-to-use wash buffer A*

This buffer is required for serum and conjugate dilution as well as washing stages.
Prior to dilution, the volume of wash buffer A must be determined for the corresponding number of tests.
First, the skimmed milk powder is dissolved in wash buffer A concentrate, and then deionised water is added to bring the solution up to the final volume (dilution: 1 + 9). The quantities required for a defined number of test strips are to be mathematically determined according to the following formula (device-specific dead volume is not considered):

Reagent	**Formula**	**Example: 5 strips**
Skimmed milk powder [g]	= number of strips x 0.1	0.5 g
Wash buffer A concentrate [ml]	= number of strips x 2	10 ml
Deionised water [ml]	= number of strips x 18	90 ml
Ready-to-use wash buffer A [ml]	= number of strips x 20	100 ml

Ready-to-use wash buffer A can be stored for 4 weeks at +2 °C to +8 °C. The ready to use wash buffer A is odourless and easily marred.

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 the 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:

Reagent	**Formula**	**Example: 5 strips**
Conjugate concentrate [µl]	= number of strips x 20	100 µl
Ready-to-use wash buffer A [ml]	= number of strips x 2	10 ml

The conjugate quantities are calculated without dead volume. Depending on handling (manual or on a device), please mix additional conjugate for 1 to 3 strips.

8 Test procedure

8.1 One-hour serum incubation

No.	**Execution**	**Note**
1	Temper all reagents for at least 30 minutes at 18°C - 25° (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 tweezers instead. 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.
b)	Incubate for **1 hour** with gentle shaking	Cover the incubation tray with plastic cover and place in the shaker.
4	Washing	Carry out washing stages 8.4a-8.4c three times in total.
a)	Carefully remove the plastic cover from the incubation trays.	Avoid cross-contamination.
b)	Gently siphon serum dilution from the individual wells.	The manufacturer's instructions must be followed during automatic processing.
c)	Pipette **2 ml** of ready to use **wash buffer A** into each well, wash for 5 minutes with gentle shaking and then siphon off the wash buffer A.	
5	Incubation with conjugate Add **2 ml** of ready-to-use **conjugate solution** and incubate for **45 minutes** while shaking gently.	Cover the incubation tray with plastic cover and place in the shaker.
6	Washing see under 8.4	Carry out washing stages three times in total (see 8.4a-8.4c)
7	Substrate reaction Add **1.5 ml** of ready-to-use **substrate solution** and incubate for **8 minutes** while shaking gently.	
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 water 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.**		

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 (top line) with clearly visible stain, dark band
2. Antibody class (second band): the IgG, IgM and/or IgA conjugate control band must show clearly visible 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 software *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 to 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 each corresponding immunoglobulin class, assess separately the intensity of the bands occurring on the basis of Table 1.

Table 1: Assessment of band intensity in relation to the cut-off band

Stain intensity of the bands	Assessment
No reaction	-
Very low intensity (lower than the cut-off band)	+/-
Low intensity (equivalent to the cut-off band)	+
High intensity (higher than the cut-off band)	++
Very strong intensity	+++

9.3 Interpretation of test results

A point assessment of Toxoplasma gondii antigens was specified in the *recom*Line Toxoplasma for safe and easy test assessment based on clinical evaluations and mathematical analysis. The test result is achieved by adding the corresponding individual point values of the bands assessed as + , ++ or +++ (Table 2). The resulting total is entered in the column with the sigma (summation) symbol. The positive, questionable or negative assessment of the sample can then be directly determined (Table 3) and entered in the assessment column.

Table 2: Point assessment of T. gondii antigens in the *recom*Line Toxoplasma

Antigen	Points in the IgG	Points in the IgM	Points in the IgA
ROP1c	1	6	4
MIC3	0	2	4
GRA7	4	4	4
GRA8	4	4	4
p30	6	0	4
MAG1	2	1	2
GRA1	4	0	4
rSAG1	4	0	4

Table 3: Assessment of the test results in the *recom*Line Toxoplasma

Points' total	Assessment
≤ 3	Negative
4 - 5	Borderline
≥ 6	Positive

9.4 Extended diagnosis by determining the avidity

The avidity determination in the *recom*Line Toxoplasma IgG can be used to extend the diagnostic results achieved with toxoplasm serology, as determining the avidity is an essential part of determining the time of infection.
Determining the avidity requires two test strips; the test must be carried out as part of the same test run:

- one test strip to determine the IgG antibody reactivity (without an avidity reagent)
- one test strip to determine the IgG avidity (with an avidity reagent)

9.4.1 *Test principle and test execution*

The avidity reagent - Item No. 11010 - may be used to determine the avidity of Toxoplasma IgG antibodies. The instructions for performing the test are given in the instructions for the use of the avidity reagent.

9.4.2 *Avidity in the recomLine Toxoplasma IgG*

The *recom*Line Toxoplasma IgG is only used to determine the avidity of the IgG antibodies for the following antigens: p30, MAG1, GRA1 and rSAG1 (the time of infection can only be delimited by determining the avidity of the IgG antibodies for these antigens). Avidity cannot be used to determine the time of infection for the antigens ROP1c, MIC3, GRA7 and GRA8.

9.4.3 *Assessment and interpretation of the avidity in the recomLine Toxoplasma IgG*

- The avidity is only determined when the overall IgG results have been positive.
- Bands on the IgG strip that have a lower reactivity than the cut-off are not taken into account when determining the avidity.
- Determining the avidity for IgG antibodies against the antigens p30, MAG1, GRA1, rSAG1.
- Compare the intensities of the corresponding bands on the two test strips (IgG strips and avidity strips) incubated with the same patient sample. Pay attention to any change in intensity.
- Low avidities may also be found for prior infections. This may be possible due to a decreasing titre, the lack of a booster effect or delayed maturity. The interpretation of avidity must always take place against the background of the overall test results, as the avidity maturity may fluctuate markedly on a case-by-case basis.

Table 4: Assessment of avidity

"High" avidity:	When the avidity is high, the band intensity of the avidity strip is hardly reduced or not reduced at all (band intensity ranging from approximately 75 to 100% of the IgG strip).
"Intermediate" avidity:	Intermediate avidity occurs when avidity cannot be clearly allocated a high or low value.
"Low" avidity:	Low avidity requires at least a 50% intensity reduction.

9.5 Notes on interpretation

The human serological immune response to a Toxoplasma gondii infection is characterised by high variability. It is especially the fact that IgM antibodies can in many cases still be detected years after an infection has occurred that renders the interpretation of serological results more difficult.

Antibody development over time:

The serological stages of Toxoplasma infection (according to Friese - modified)

<table>
<tr><th></th><th>Infection phase</th><th>Period</th><th>Typical progression of immune response (according to Friese et al.)</th></tr>
<tr><td rowspan="2">Phase I</td><td>Sero-conversion or significant titre increase</td><td>0 – 3 months p.i.</td><td>After 10 - 14 days, specific antibodies usually occur in the sequence IgM, IgG, IgA.
High concentrations of specific IgM antibodies accompanied by a lack or low number of positive IgG antibodies give rise to a suspicion (but not proof) of an acute infection.</td></tr>
<tr><td colspan="3">Typical progression of IgG immune response of Mikrogen recombinant antigens:
During the early sero-conversion phase of the infection, antibodies against GRA7 and/or GRA8 will be the first to occur. This will be followed by antibodies against p30.
This is in turn followed by antibodies against MAG1 and GRA1, which in Phase I will be mainly detected at low or, in some cases, intermediate avidity.
Where only IgG antibodies with low or intermediate avidity and/or no IgG antibodies against the antigens p30, MAG1, GRA1 and rSAG1 can be detected, an acute infection may be suspected, with the infection classified as "Phase I". A follow-up test is strongly recommended to document a possible titre increase.
High-avidity antibodies against p30 are detected towards the end of Phase I.
Note: The detection of high-avidity antibodies against p30 indicates an infection of more than 2 months ago.
In some cases, antibodies against rSAG1 with low avidity may be found at the end of Phase I.
Remark on IgM: In the event of an acute infection, the IgM results will usually be positive. In rare cases, an acute Toxoplasma infection may also be linked to very low IgM titres or even to a complete lack of detectable IgM antibodies (IgM "low responder"). Where medication has been administered in the early stages, the antibody titres - especially the IgM titre - will usually drop faster.</td></tr>
<tr><td rowspan="2">Phase II</td><td>Active infection</td><td>3 – 6 months p.i.</td><td>Achieving maximum antibody production. Usually medium to high concentrations of IgM, IgG and IgA antibodies can be detected. The follow-up results will no longer indicate any titre increases.</td></tr>
<tr><td colspan="3">Typical progression of IgG immune response of Mikrogen recombinant antigens:
High-avidity antibodies against MAG1 and/or GRA1 occur first during Phase II.
Definition: The detection of high-avidity antibodies against MAG1 and/or GRA1 excludes an infection in Phase I.
This assessment is independent of p30 avidity. This is followed by low-avidity or intermediate-avidity antibodies against rSAG1.
In rare cases, high-avidity antibodies against rSAG1 can be detected.
GRA7 and/or GRA8 are detected in high concentrations; the avidity of these antibodies is not assessed.
Remark on IgM: The IgM results are usually positive.</td></tr>
<tr><td rowspan="2">Phase III</td><td>Subsiding (sub-acute) infection</td><td>6 – 12 (-36) months p.i.</td><td>Gradually subsiding antibody concentrations, usually in the sequence IgM, IgA, IgG.</td></tr>
<tr><td colspan="3">Typical progression of IgG immune response of Mikrogen recombinant antigens:
High-avidity antibodies to rSAG1 are detected first in Phase III.
Definition: The detection of high-avidity antibodies against rSAG1 indicates an infection that usually occured more than 6 months ago. This applies independently of the occurrence or the avidity of antibodies against p30, MAG1 and/or GRA1.
In rare cases, high-avidity rSAG1 may also occur towards the end of Phase II.
GRA7 and/or GRA8 are detected in high concentrations; the avidity of these antibodies is not assessed.
Remark on IgM: The IgM results are usually questionable to positive.</td></tr>
</table>

<table>
<tr><td rowspan="2">Phase IV</td><td>Latent infection</td><td>> 12 months p.i.</td><td>IgM and IgA antibodies can no longer be detected. Usually low to medium IgG antibody concentration. Immune protection, no risk of connatal Toxoplasma infection.</td></tr>
<tr><td colspan="3">Typical progression of IgG immune response of Mikrogen recombinant antigens:
High-avidity antibodies to rSAG1 are detected first in Phase IV.
The detection of high-avidity antibodies against rSAG1 indicates an infection that usually occured more than 6 months ago. This applies independently of the occurrence or the avidity of antibodies against p30, MAG1 and/or GRA1.
A delimitation between Phase IV and Phase III is only possible via the IgM status (see remark on IgM)
The antibody concentration against GRA7 and/or GRA8 can drop below the detection limit in the case of infections that occurred a very long time ago. The avidity of these antibodies is not assessed.
Remark on IgM: IgM and IgA antibodies can usually no longer be detected, but IgM antibodies may sometimes persist for a very long time (up to several years).</td></tr>
</table>

9.5.1 *IgG antibody response:*
The IgG response is particularly characterised by GRA7, GRA8, p30, MAG1, GRA1 and rSAG1. While IgG antibodies against GRA7 and/or GRA8 are normally already detected at the beginning of Phase I, followed by p30, IgG antibodies against MAG1 and GRA1 only occur somewhat later. Antibodies against rSAG1 usually only develop in Phase II.
When the infection occurred a long time ago, it is possible that the antibody titre will drop below the detection limit. The most reliable marker for prior infections is the p30 antigen. An isolated p30 reactivity for IgG with a simultaneously negative IgM indicates that an infection occurred a long time ago.
Questionable IgG results do not constitute an adequate criterion for immunity to Toxoplasma infections.

9.5.2 *IgG avidity:*
The following antigens are used to evaluate avidity: p30, MAG1, GRA1 and rSAG1.
In most cases, determining the avidity for individual antigens makes it possible to determine the infection status more accurately. This is particularly important for distinguishing between an acute and a subsiding Toxoplasma infection with persistent IgM antibodies (see 9.4). The fact that the IgG antibody reactions against specific antigens typically start in various phases of the infection and that these IgG antibodies successively mature from low-avidity to high-avidity usually makes a differentiated view of the infection status possible by analysing the IgG bands and avidity patterns. The typical development of the IgG response to a Toxoplasmosis infection is described below, but there may be individual deviations from the typical progression.
Important:
Where a patient sample shows high-avidity antibodies against several avidity antigens, time allocation always takes place on the basis of the antigen that indicates the latest period (phase) of infection:

Table 5: Assessment of avidity

No high avidity for p30, MAG1, GRA1, rSAG1	high avidity against p30#	high avidity against MAG1 and/or GRA1	high avidity against rSAG1*
Susp. < 3 months p.i.	Susp. > 2 months p.i.	Susp. > 3 months p.i.	Susp. > 6 months p.i.*
Phase I		**Phase II**	**Phase III**

In rare cases, high-avidity rSAG1 may also occur after 4 months (Phase II).
Proof of isolated IgG reactivity against p30 with high avidity but no proof of further IgG bands, with a simultaneously negative IgM, indicates a prior Toxoplasma infection.

Please note:
To determine the time of infection with the aid of the avidity antigens, it is very important that only clearly high avid reactions should be used in the assessment. Intermediate avidities are to be allocated to the "low-avidity" interpretation pattern.
The avidity maturity of the various antigens and the development of the immune response can be delayed and/or not take place at all, especially when anti-parasitic treatment has been administered.
When infections have occurred a long time ago, the decreasing IgG antibody titres and the lack of a booster effect may yield false low-avidity results.
Deviations of the progression of the avidity development from these typical constellations are possible and require a particularly critical interpretation, especially in the case of pregnancy-relevant infections.

9.5.3 *IgM antibody response:*
In the event of an acute infection, the IgM results will usually be positive. In rare cases, an acute Toxoplasma infection may also be linked to very low IgM titres or even to a complete lack of detectable IgM antibodies (IgM "low responder").
Where medication has been administered in the early stages, the antibody titres - especially the IgM titre - will usually drop faster.
The main marker of the IgM response in the *recom*Line Toxoplasma is the ROP1c antigen. Other IgM antigens are MIC3, GRA7 and GRA8. Antibodies against several IgM antigens may be detected during the acute phase of the infection. IgM antibodies against ROP1c almost always occur during the early phase of the infection and may be responsible for the persistence of the IgM titre.
MIC3 is another IgM-specific marker, but does not have the immunodominance of ROP1c and is therefore not detected as frequently. A positive antibody reactivity against MIC3 antigens may, however, support the IgM results, especially when there is a lack of ROP1c reactivity.
During the acute phase of the infection, IgM antibodies against GRA7 and/or GRA8 may also be frequently detected, but these may often persist after the acute infection phase.
Isolated IgM results without the detection of IgG antibodies must be particularly carefully assessed, as these may be sero-conversions. Possible unspecific reactions (e.g. as a result of polyclonal stimulation) cannot be excluded, however. For this reason, a follow-up test is required at intervals of 2-3 weeks.

9.5.4 *IgA antibody response:*
The IgA immune response may vary a great deal overall. On the one hand, there may be no such response at all, but, on the other hand, the presence of IgA antibodies may substantiate the suspicion of an acute Toxoplasmosis infection [4]. However, an analysis of the IgA band pattern cannot be used to derive any information about the status of the infection.

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.
- Samples with inconclusive or questionable results should be followed up after 2-3 weeks, subject to the clinical situation.
- A negative result does not entirely exclude the possibility of a toxoplasma infection. False negative results can occur if the sampling is made before the initial reaction of the immune system.
- Where the serum test results are negative for a pregnant woman, a follow-up sample should be taken and tested after 8-12 weeks.
- Where the band constellations are uncertain, further monitoring is recommended.
- It must also be taken into account that treatment may result in delayed IgG and/or IgM antibody formation, thus also influencing the IgG avidity.
- Deviations of the progression of the immune response from these typical constellations are possible and require particularly careful interpretation.
- For all test interpretations, especially in the case of slightly positive results, the incorporation of possible clinical information is essential. Once again, close cooperation between the laboratory and the attending physician is recommended.
- Dark test strips: Some patient samples can produce 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

The diagnostic sensitivity was calculated on the basis of a total of 60 clinically defined samples taken from pregnant women, with the infection time being between 0-3 months p.i. (Panel 1) and/or 3-6 months p.i. (Panel 2).

Table 6: Diagnostic sensitivity

	*recom*Line Toxoplasma IgG	*recom*Line Toxoplasma IgM
Diagnostic sensitivity %(n)	100% (60/60)[1]	90% (54/60)[2]

[1] Two samples for which the recomLineToxoplasma yielded questionable results were counted among the positive results.
[2] Nine samples for which the recomLineToxoplasma yielded questionable results were counted among the positive results.

11.2 Diagnostic specificity

The diagnostic specificity (no symptoms, comparative test results negative) is calculated on the basis of 20 samples:

Table 7: Serum collective: Without symptoms, seronegative in the screening test

	*recom*Line Toxoplasma IgG	*recom*Line Toxoplasma IgM
Diagnostic specificity %(n)	100% (20/20)	90% (18/20)[1]

[1]For two samples, the *recom*Line Toxoplasma test yielded questionable results.

11.3 Diagnostic sensitivity of phase- and band-specific avidity

11.3.1 *Infection during the last three months, patient group: Pregnant women*

Table 8: Forty-four samples of sero-conversions and progressions with a significant titre increase (indicative of an acute infection). See samples from 11.1, Panel 1.

IgG-positive samples	Overall assessment	Number
Phase I	Susp. < 3 months p.i.	41
Phase I	Susp. > 2 months p.i.	0
Phase II	Susp. > 3 months p.i.	0
Phase III	Susp. > 6 months p.i.	0
Total samples		41[1]

[1]For three samples, the *recom*Line Toxoplasma IgG yielded questionable results (no determination of avidity possible)
All IgG-positive samples can be clearly allocated to the acute phase (< 3 months p.i.).

11.3.2 *Infection 3 to 6 months ago, patient group: Pregnant women*

Table 9: The 16 samples of the sero-group for 11.1. (Panel 2) are follow-up tests for Panel 1.

IgG-positive samples	Overall assessment	Number
Phase I	Susp. < 3 months p.i.	7
Phase I	Susp. > 2 months p.i.	5
Phase II	Susp. > 3 months p.i.	4
Phase III	Susp. > 6 months p.i.	0
Total samples		16

None of the follow-up tests indicated a band sample in Phase III (>6 months p.i.).

11.3.3 *Subsiding infection, patient group: Pregnant women*

Table 10: Ninety-four individual samples with a low to medium IgG titre and a positive, questionable or negative IgM result.

IgG-positive samples	Overall assessment	Number
Phase I	Susp. < 3 months p.i.	16
Phase I	Susp. > 2 months p.i.	14
Phase II	Susp. > 3 months p.i.	42
Phase III	Susp. > 6 months p.i.	22
Total samples		94

11.3.4 *Infection long ago, patient group: Blood donor*

Table 11: 126[5] samples from blood donors

IgG-positive samples	Overall assessment	Number
Phase I	Susp. < 3 months p.i.	13
Phase I	Susp. > 2 months p.i.	21
Phase II	Susp. > 3 months p.i.	14
Phase III	Susp. > 6 months p.i.	19
Total samples		67

[5] Fifty-three of the 126 samples yielded a negative IgG result, 6 samples yielded a questionable IgG result.

The blood donor samples showed the effect that false low-avidity results could be obtained for infections that occurred long ago, due to reduced antibody titres and a missing booster effect.

11.3.5 *Routine serums with a positive preliminary IgG result for Toxoplasma, patient group: Routine laboratory samples taken from pregnant women*

Table 12: Eighty-nine samples with preliminary positive IgG results for Toxoplasma

IgG-positive samples	Overall assessment	Number
Phase I	Susp. < 3 months p.i.	3
Phase I	Susp. > 2 months p.i.	21
Phase II	Susp. > 3 months p.i.	23
Phase III	Susp. > 6 months p.i.	42
Total samples		89

This serum group showed that, unlike the tested blood donors, the percentage of samples for which an infection time more than 6 months ago could be allocated on the basis of avidity was significantly higher. On average, this patient group had significantly higher avidities (especially for rSAG1) than the blood donors, most probably due to the age structure (pregnant women 20-40 years, blood donors 18-65 years).

11.4 Sero-prevalence

	*recom*Line Toxoplasma IgG		*recom*Line Toxoplasma IgM	
	Seropositive %(n)	Seronegative %(n)	Seropositive %(n)	Seronegative %(n)
Blood donor	58% (73/126)[1]	42% (53/126)	8% (10/128)[2]	92% (118/128)

[1] Six samples that yielded questionable results for rLTG V03 were counted among the positive results.
[2] Five samples that yielded questionable results for rLTG V03 were counted among the positive results.

11.5 Analytical specificity

The analytical specificity is defined as the capacity of the test to precisely determine the analytes in the presence of potential interference factors in the sample matrix or cross-reactions with potentially interfering antibodies.
a) Interferences: Control studies on potentially interfering factors have shown that the test is not affected by anticoagulants (sodium citrate, EDTA, heparin), hemolysis, triglycerides or bilirubinaemia of the sample. Lipemic sera can cause interferences in the *recom*Line Toxoplasma IgG.
b) Cross-reactions: In control studies, the potential interferences of antibodies against other organisms (e.g. EBV*) are examined. Also tested were conditions caused by atypical activity of the immune system (antinuclear autoimmune antibodies**, rheumatoid factor**). There was no evidence of cross-reactions.
* Acute EBV infections may cause a nonspecific IgM reactivity in the *recom*Line Toxoplasma IgM (e.g. polyclonal stimulation).
** Samples, which have antinuclear antibodies and rheumatoid factors, may cause interferences in the *recom*Line Toxoplasma IgG.

12 Literature

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We would be glad to send you further literature on the diagnosis of toxoplasmosis on request.

13 Explanation of symbols

Symbol	Meaning
Σ	Content is sufficient for <n> applications Number of applications
WASHBUF A 10 X	Wash Buffer A (10 times concentration)
SUBS TMB	Chromogenic substrate Tetramethylbenzidin
MILKPOW	Skimmed milk powder
TESTSTR	Test strips
CONJ IgG	Anti-human IgG conjugate
AVIDI	Avidity reagent
ADD	Additional reagent, available on request
CONJ IgA	Anti-human IgA conjugate
CONJ IgM	Anti-human IgM conjugate
EVALFORM	Evaluation form
INSTRU	Instructions for use
i	See instructions for use
CONT	Contents, includes
IVD	In vitro test
LOT	Batch/version number
	Do not freeze
REF	Order number
	Use by Expiry date
x°C y°C	Store at x°C to y°C
	Manufacturer

14 Manufacturer and version information

***recom*Line Toxoplasma IgG [Avidität]** ***recom*Line Toxoplasma IgM [IgA]**	Item no. **5972** Item no. **5973**
Instructions for use valid from	GARLTG011EN 2023-03
MIKROGEN GmbH Anna-Sigmund-Str. 10 82061 Neuried Germany Tel. +49 89 54801-0 Fax +49 89 54801-100 E-mail mikrogen@mikrogen.de Internet www.mikrogen.de	
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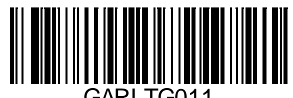
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