*recom*Line Chlamydia IgG
*recom*Line Chlamydia IgA [IgM]

MIKROGEN DIAGNOSTIK

IVD

Instructions for use (English)

1 Purpose

The *recom*Line Chlamydia IgG, IgA [IgM] is a qualitative in vitro test for the detection of IgG, IgA or IgM antibodies against *Chlamydia trachomatis, Chlamydia pneumoniae* and *Chlamydia psittaci* in human serum or plasma.

2 Intended use

The *recom*Line Chlamydia IgG, IgA [IgM] is a line immunoassay. In contrast to ELISA, the test principle allows the identification of specific antibodies against the various antigens of *Chlamydia trachomatis* (MOMP, OMP2, TARP, CPAF, HSP60)*, Chlamydia pneumoniae* (MOMP, OMP2, TARP, CPAF, YwbM) und *Chlamydia psittaci (*MOMP, OMP2, TARP, CPAF) through the separate line-up of the individual antigens. The *recom*Line Chlamydia IgG, IgA [IgM] is a confirmatory test and can be used to clarify unclear screening results.

3 Test principle

Highly purified recombinant Chlamydia 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 stage, the strips are incubated with anti-human immunoglobulin antibodies (IgG, IgA and/or IgM), which are coupled to horseradish 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 band under the strip number, which must show a reaction for every serum/plasma sample.
b) The conjugate controls (IgG, IgA, IgM) are used for the inspection of the antibody class detected. If, for example, the test strip is used for the detection of IgG antibodies, the IgG conjugate will show this clearly on the band.
c) "Cutoff control": The intensity of this band allows the assessment of the reactivity of each of the antigen bands (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 skim milk powder
INSTRU	**1** Instructions for use
EVALFORM	**1** Evaluation form

4.1.1 *recomLine Chlamydia IgG*

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)** obtained from rabbits, contains NaN3 (<0.1%), MIT (<0.1%) and chlorazetamide (<0.1%)

4.1.2 *recomLine Chlamydia IgA [IgM]*

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

TESTSTR	**2** tubes, each with 10 numbered test strips
CONJ IgA	**500 µl** anti-human IgA conjugate **(100-fold concentration, colourless cap)** obtained from rabbits, contains NaN3 (<0.1%), MIT (<0.1%) and chlorazetamide (<0.1%)

Also available for the determination of IgM antibodies (in addition to *recom*Line Chlamydia IgA [IgM]):

CONJ IgM	**500 µl** anti-human IgM conjugate **(100-fold concentration, purple cap)** obtained from rabbits, contains NaN3 (<0.1%), MIT (<0.1%) and chlorazetamide (<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.
- Wash buffer, milk powder, dilution buffer, conjugates and TMB can be exchanged between different recomLine and/or recomBlot test systems if these components have the same symbol. Special attention should be paid to the expiry dates 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 substantial changes to the product or the regulations concerning 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 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 produced with inactivated whole-cell lysates and/or recombinant bacterial, viral or parasite antigens.
- After adding the patient or control material, the strips must be regarded as potentially infectious and be treated correspondingly.
- Suitable disposable gloves must be worn throughout the entire test procedure.
- The reagents contain the antimicrobial agents and preservatives sodium azide (NaN3), MIT (methylisothiazolinone), oxypyrion, chloroacetamide and hydrogen peroxide. Avoid contact with the skin or mucous membrane. Sodium azide (NaN3) can form an explosive azide upon contact with heavy metals such as copper and lead.
- All siphoned liquids must be collected. All collective containers must contain suitable disinfectants for the inactivation of human pathogens and be autoclaved. All reagents and materials contaminated with potentially infectious samples must be treated with appropriate 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.
- 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. Prolonged storage of the samples is possible at - 20°C or below. 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

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 a) b)	Incubation of samples **20 µl** of undiluted sample (human serum or plasma) is pipetted on to the test strip for each incubation mixture. (Dilution 1 + 100) Incubate for **1 hour** with gentle shaking	 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 plastic cover and place in the shaker.
4 a) b) c)	Washing Carefully remove the plastic cover from the incubation trays. Gently siphon serum dilution from the individual wells. 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.	Carry out washing stages 8.4a-8.4c three times in total. Avoid cross-contamination. The manufacturer's instructions must be followed during automatic processing.
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 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 **deionisied 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, third and fourth bands): the IgG, IgA and / or IgM conjugate control band must show a clear staining.
3. Cut-off control (fifth 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	+++

Caution!
The band patterns in the *recom*Line Chlamydia IgG, IgA and IgM detection can show different intensities. It is possible that the *recom*-Line Chlamydia IgG shows stronger and darker bands than the *recom*-Line IgA or IgM. The intensity of the protein bands is dependent on the concentration of the Chlamydia-specific antibodies

9.3 Interpretation of test results

The test result is determined by adding the point values according to Table 2 the various reactive ≥ cut-off bands (i.e. using bands rated at least as +). The resulting sum is entered in the column with the sum symbol.
The positive, inconclusive or negative assessment of the sample can then be determined directly with the Table 3 and entered in the assessment column of the evaluation sheet.

Table 2: Point assessment of Chlamydia antigens

	Point assessment for								
Antigen	***Chlamydia trachomatis* Antigens**			***Chlamydia pneumoniae* Antigens**			***Chlamydia psittaci* Antigens**		
	IgG	IgA	IgM	IgG	IgA	IgM	IgG	IgA	IgM
MOMP	6	6	3	6	6	3	4/6**	6	6
OMP2	2	6	3	2/6*	6	3	1	4	3
TARP	3	4	3	3	4	3	3	4	3
CPAF	3	6	3	3	6	3	3	6	3
HSP60	1	3	3	-	-	-	-	-	-
YwbM	-	-	-	6	6	3	-	-	-

* 6 points if the OMP2 of *C. trachomatis* and *C. psittaci* are negative, otherwise 2 points.
** 6 points if the MOMP of *C. trachomatis* and of *C. pneumoniae* are negative, otherwise 4 points

Table 3: Interpretation of the results in *recom*Line Chlamydia

Points' total	Assessment
≤ 3	negative
4 - 5	borderline
≥ 6	positive

10 Limitations of the method - restrictions

- Serological test results must always be seen in the context of the clinical picture of the patient. Therapeutic consequences of the serological findings must always be taken in context with the clinical data.
- A negative test result for *recom*line Chlamydia IgG, IgA [IgM] cannot exclude an infection with Chlamydia spec. If there is persistent suspicion of an infection with Chlamydia , a further sample should be taken and tested after two weeks.
- A positive *recom*Line Chlamydia IgG, IgA [IgM] test result does not necessarily mean that there is an active illness event.
- In the interpretation of the serological results, it is indispensable to include the case history, clinical symptoms and additionally available laboratory data in the total diagnosis. Thus, a Chlamydia spec. infection is probable when chlamydia antibodies are first detected and there are clear clinical symptoms. A second sample should be taken two weeks later to clarify the increase in antibodies.
- In the event of an infection with the Epstein-Barr virus (EBV), polyclonal stimulation of B-lymphocytes may be used to trigger an unspecific reaction once antibodies in the IgA class have been detected. It is recommended to exclude an EBV infection by means of a differential diagnosis if the results are positive or borderline.
- 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 Sensitivity

Table 4: Test results of samples positive in two reference assays

	C. trachomatis		C. pneumoniae		C. psittaci	
	IgG (n=82)	IgA (n=39)	IgG (n=82)	IgA (n=20)	IgG (n=8)	IgA (n=8)
positive	80	37	80	20	6	8
borderline	2	2	1	0	2	0
negative	0	0	1	0	0	0
sensitivity (%)	100*	100*	99*	100	100*	100

* incl. borderline results
Due to very low number of defined IgM positive samples, no sensitivity has been calculated.

11.2 Specificity

Table 5: Test results of samples negative in two reference assays

	C. trachomatis			C. pneumoniae			C. psittaci		
	IgG (n=110)	IgA (n=134)	IgM (n=137)	IgG (n=51)	IgA (n=87)	IgM (n=137)	IgG (n=93)	IgA (n=96)	IgM (n=137)
negative	110	134	137	51	87	137	93	96	137
borderline	0	0	0	0	0	0	0	0	0
positive	0	0	0	0	0	0	0	0	0
specificity (%)	100	100	100	100	100	100	100	100	100

11.3 Prevalence

Tabelle 6: Test results of 100 Blood Donors

	C. trachomatis			C. pneumoniae			C. psittaci		
	IgG	IgA	IgM	IgG	IgA	IgM	IgG	IgA	IgM
positive	15	3	0	41	4	0	0	0	0
borderline	5	0	0	0	1	0	0	1	0
negative	80	97	100	59	95	100	100	99	100
prevalence (%)	15	3	0	41	4	0	0	0	0

11.4 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 (e.g. anticoagulants, haemolysis, effects of the sample handling) or cross reactions with potentially interfering antibodies.
a) Interferences: Control studies on potentially interfering factors have shown that anticoagulants (sodium citrate, EDTA, heparin), haemolysis, lipaemia or bilirubinaemia or cycles of freezing and thawing do not affect the performance of the test. In hemolytic samples an increased number of positive *Chlamydia trachomatis* IgG results were observed, isolated antibody responses against *C. trachomatis* and *C. pneumoniae* were observed in IgA.
b) Cross-reactions: The potential interference of antibodies with other organisms (e.g. EBV, CMV, *Treponema pallidum* and *Bordetella pertussis*) has been investigated in control studies. Also tested were conditions caused by atypical activity of the immune system (antinuclear autoimmune antibodies, rheumatoid factor, pregnancy). The serum of patients infected with *Treponema pallidum* demonstrated an increased number of positive results for *Chlamydia trachomatis*, probably because of a co-infection with both pathogens. In patient samples with acute EBV and ANA-positive results an increased number of *C. pneumoniae* IgA results was observed. In rheumatoid factor positive samples increased *C. pneumoniae* IgG results have been observed. In IgA isolated antibodies against all three Chlamydia species could be detected. In the other groups no cross-reactivities have been detected.

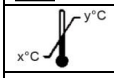
12 Literature

1. K.D. Everett, R.M. Bush and A.A. Andersen: Emended description of the order Chlamydiales, proposal of Parachlamydiaceae fam. nov. and Simkaniaceae fam. nov., each containing one monotypic genus, revised taxonomy of the family Chlamydiaceae, including a new genus and five new species, and standards for the identification of organisms. *Int J Syst Evol Microbiol* 2001, 51 (1), 249, 251-253
2. M. Askienazy-Elbhar and J. Henry-Suchet: Persistent „silent" *Chlamydia trachomatis* female genital tract infections. *Infect Dis Obstet Gynecol* 1999, 7, 31-34
3. I. Sziller, S.S. Wittkin, M. Ziegert, Z. Csapo, A. Ujhazy and Z. Papp: Serological responses of patients with ectopic pregnancy to epitopes of the *Chlamydia trachomatis* 60 kD heat shock protein. *Hum Reprod* 1998, 13, 1088-1093
4. R. Andrie, P. Braun, U. Welsch, E. Straube, W. Höpp, E. Erdmann, B. Lüderitz and G. Bauriedel: *Chlamydiales* und humanes Hitzeschockprotein 60 bei akutem Koronarsyndrom Antikörpervermittelte (Auto-) Immunreaktion

als link zwischen Infektion und Arteriosklerose. *Z Kardiol* 2003, 92 (6), 455-465
5. O. Burkhardt, E. Straube and T. Welte: Clinical picture, diagnosis and treatment of *Chlamydia pneumophila. Pneumonolgie* 2003, 57 (8), 449-458
6. S. Bas, P. Muzzin, B. Ninet, J.E. Bornand, C. Scieux and T. L. Vischer: Chlamydial Serology: Comparative Diagnostic Value of Immunoblotting, Microimmunefluorescence Test, and Immunoassays Using Different Recombinant proteins as Antigens: *J Clin Microbiol* 2001, 39 (4), 1368-1377
7. A. J. Littman, L. A. Jackson, E. White, M. D. Thornquist, C. A. Gaydos and T. L. Vaughan: Interlaboratory Reliability of Microimmuno-fluorescence test for Measurement of *Chlamydia pneumoniae*-Specific Immuno-globulin A and G Antibody Titers. *Clin. Diagn. Lab. Immunolo.* 2004, 11 (3), 615-617
8. S. Bunk, I. Susnea, J. Rupp, JT. Summersgill, M. Maass, W. Stegmann, A. Schrattenholz, A. Wendel, M. Przybylski, C. Hermann: Immunoproteomic identification and serological responses to novel Chlamydia pneumoniae antigens that are associated with persistent C. pneumoniae infections. J Immunol. 2008 Apr 15;180(8):5490-8
9. F. Radouani , J. Maile , F. Betsou: Serological profiling with Chlamycheck, a commercial multiplex recombinant antigen Western blot assay of chlamydial infections. Can J Microbiol. 2007 Dec;53(12):1360-8

We will gladly send you further literature on the diagnosis of Chlamydia on request.

13 Explanation of symbols

	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
CONJ IgA	Anti-human IgA conjugate
ADD	Additional reagent, available on request
CONJ IgM	Anti-human IgM conjugate
EVALFORM	Evaluation form
INSTRU	Instructions for use
	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
	Store at x°C to y°C
	Manufacturer

14 Manufacturer and version information

***recom*Line Chlamydia IgG** ***recom*Line Chlamydia IgA [IgM]**	Item no. **6172** Item no. **6173**
Instructions for use valid from	GARLCY012aEN 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	
	CE 0483

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