*recom*Well Chlamydia trachomatis IgG
*recom*Well Chlamydia trachomatis IgA

MIKROGEN DIAGNOSTIK

IVD

Instructions for use (English)

1 Purpose

The *recom*Well Chlamydia trachomatis IgG, IgA test is a qualitative and quantitative in-vitro test for the detection and identification of IgG or IgA antibodies against *Chlamydia trachomatis* in human serum or plasma. The *recom*Well Chlamydia trachomatis IgG, IgA is an indirect sandwich ELISA method.

2 Intended use

The *recom*Well Chlamydia trachomatis test is used to detect the presence of IgG and IgA antibodies to the proteins MOMP, TARP and CPAF.
This serological test provides information about the infection status, especially in the case of chronified, ascending or inapparent infections. IgA antibodies occur within 2 to 4 weeks, while IgG antibodies occur after approx. 4 weeks. While IgG antibody titres decrease only gradually and may persist throughout life, IgA antibodies will disappear approximately 6 months after the healing process (for example, following treatment with antibiotics). IgA antibodies can occur in primary, chronic and recurring infections, as can an increase in the IgG titres.

3 Test principle

Highly purified Chlamydia trachomatis antigens (MOMP, TARP and CPAF) are fixed in the cavities of the micro-titre plate.

1. Diluted serum or plasma samples are incubated in the wells, with antibodies binding specifically to the pathogen antigens coating the surface of the wells.
2. Unbound antibodies are then flushed away.
3. In a second step, anti-human immunoglobulin antibodies (IgG and/or IgA), which are coupled to horseradish peroxidase, are incubated in the wells.
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 occurs, the chromogen substrate solution colours proportionally to the quantity of the bound anti-Chlamydia trachomatis IgG or IgA antibodies. The intensity of the colour development can be measured using a photometer. The concentration of the anti-Chlamydia trachomatis antibodies in the sample can be deduced in this way.

4 Reagents

4.1 Package contents

The reagents in one package are sufficient for 96 tests.
Each test kit contains:

WASHBUF 10 X	**100 ml** Wash buffer (**ten times concentration**) Contains phosphate buffer, NaCl, detergent, preservative: MIT (0.01%) and Oxypyrion (0.1%)
DILUBUF	**125 ml** Dilution Buffer (**ready-to-use**) Contains protein, detergent and blue dye. Preservative: MIT (0.01%) and Oxypyrion (0.1%)
SUBS TMB	**12 ml** Chromogenic substrate tetramethylbenzidine (TMB, **ready-to-use**)
SOLN STOP	**12 ml** stop solution 24.9% phosphoric acid (H_3PO_4) (**ready for use**)
INSTRU	**1** Instructions for use
EVALFORM	**1** Evaluation form
TAPE	**2 pieces** of covering film

***recom*Well Chlamydia trachomatis IgG** also contains the following:

MTP	**12X8 wells** Microtitre plate (cap strip marked in **red**) coated with recombinant Chlamydia trachomatis antigens in the self-sealing vacuum bag.
CONTROL + IgG	**450 µl** positive control (**violet** cap) contains MIT (0.1%) and Oxypyrion (0.1%)
CONTROL ± IgG	**450 µl** cutoff control (**yellow** cap) contains MIT (0.1%) and Oxypyrion (0.1%)
CONTROL - IgG	**450 µl** negative control (**white** cap) contains MIT (0.1%) and Oxypyrion (0.1%)
CONJ IgG	**500 µl** anti-human IgG conjugate (**101-times concentrated**, **red** cap) contains NaN_3 (<0.1%), MIT (<0.01%) and chlorazetamide (<0.1%)

***recom*Well Chlamydia trachomatis IgA** also contains the following:

MTP	**12X8 wells** Microtitre plate (cap strip marked in **blue**) coated with recombinant Chlamydia trachomatis antigens in the self-sealing vacuum bag.
CONTROL + IgA	**450 µl** positive control (**brown** cap) contains MIT (0.1%) and oxypyrion (0.1%)
CONTROL ± IgA	**450 µl** cut-off control (**orange** cap) contains MIT (0.1%) and oxypyrion (0.1%)
CONTROL - IgA	**450 µl** negative control (**white** cap) contains MIT (0.1%) and Oxypyrion (0.1%)
CONJ IgA	**500 µl** anti-human IgA conjugate (**101-times concentrated**, **blue** cap) contains NaN_3 (<0.1%), MIT (<0.01%) and chlorazetamide (<0.1%)

4.2 Materials required but not supplied

- Deionised water (high quality)
- Test tube
- Vortex mixer or other rotators
- 8-channel pipette or washer with pump
- Clean measuring cylinders, 50 ml and 1000 ml
- Micropipettes with disposable tips, 10 µl and 1000 µl
- 10 ml pipette or dispenser
- Incubation chamber 37 °C
- Microtitre plate photometer
- Timer
- 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 components' dilution buffer, wash buffer, substrate and stop solution for the *recom*Well test can be used across the whole range of parameters and batches. At the same time, the shelf life of these components is to be noted.
- The control serums and conjugates are batch-dependent and may not be used across the whole range of parameters or batches.
- Mix the concentrated conjugates, controls and patient samples thoroughly before use. Avoid the build-up of foam.
- All MIKROGEN microtiter plates are equipped with Break-a-part-bars
- The covering films are intended for single use only.
- The packages bear an expiry date. Once 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 the patient samples and conjugate solution carefully. Make sure that incubation solutions do not flow over into other wells.
- 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 microtitre wells have been coated with inactivated whole cell lysates, bacterial or viral antigens.
- After the addition of patient or control specimens, the microtitre wells must be considered to be potentially infectious and handled accordingly.
- Donors' blood, in which no antibodies against HIV 1/2, HCV and hepatitis Bs antigen have been detected, is used for the manufacture of the control material. The control material must be treated with the same care as a patient sample, as infection cannot be excluded with total certainty.

- Suitable disposable gloves must be worn throughout the entire test procedure.
- The conjugates contain the antimicrobial agents and preservatives sodium azide, MIT (methylisothiazolone), oxypyrion, chloroazetamide 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.
- Phosphoric acid is an irritant. It is mandatory to avoid contact with skin and mucous membranes.
- All fluids to be disposed of 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.
- Only use microtitre wells once.
- 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

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

The test reagents are sufficient for 96 test runs. The following quantity specifications relate to the processing of a single microtitre plate strip with 8 wells respectively. While using several microtitre plate strips, the specified quantities must be simultaneously multiplied with the number of used microtitre plate strips respectively. The device-specific dead volume must be taken into account. The dilution buffers, substrate and stop solution are ready to use.

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

The wash buffer concentrate is diluted **1 + 9** with H_2O (deionised water). 5 ml concentrate is mixed with 45 ml H_2O (deionised water) per microtitre plate strip with 8 wells. The ready-to-use wash buffer can be stored for four weeks at +2 ºC to +8 ºC or a week at room temperature.

7.2.2 *Preparation of conjugate solution*

For each microtitre plate strip with 8 wells, 1 ml of dilution buffer and 10 µl of anti-human IgG peroxidase conjugate (red cap) are transferred to a clean container and mixed well (dilution **1 + 100**). The conjugate solution must be prepared <u>just before use</u>. It is not possible to store the ready-to-use conjugate solution.

8 Test procedure

No.	Execution	Note
1	Expose all reagents for at least 30 minutes to 18-25 °C (room temperature) before beginning the test.	Bring the microtitre plate to room temperature **in a sealed bag**, to avoid condensation of water. Following the removal of the required strips, the plate must be resealed in the bag and stored in the refrigerator. Before use, mix the control and patient serums, as well as the concentrated conjugates thoroughly and then centrifuge briefly if possible, so as to collect the fluid at the bottom of the containers.
2	<u>Preparing samples and controls</u> Pipette 10 µl sample and / or control to every **1 ml** dilution buffer and mix well (dilution **1 + 100**).	**The dilution of the samples and controls must always be performed just before carrying out the test.** For each test step, all of the controls must be carried out, diluted just like the patient samples.
3	<u>Incubation of samples</u> Pipette **100 µl** of diluted sample and/or diluted control into each well and incubate for **1 hour** at +**37 °C**.	Assign at least one value from the negative control, positive control and patient samples. The cutoff control must be assigned twice. Preferably a cutoff control should be included at the beginning of the series and at the end of the series respectively. In manual processing, carefully cover tightly the microtitre plate with unused cover film. Use the incubation chamber at + 37 °C.
4	<u>Washing</u>	It is recommended to carry out this step with a corresponding ELISA wash device. It is mandatory to ensure that the wash buffer is completely removed between the washing steps.
a)	Carefully remove the covering film.	
b)	Completely empty the wells	Suck off or pour out and beat out the contents.
c)	Fill each of the wells with **300 µl** of ready-to-use wash buffer → (8.4b)	Carry out the washing steps 8.4b and 8.4c **four times** in total.
5	<u>Incubation with conjugate</u> Add **100 µl** of diluted conjugate solution (7.2.2) and incubate for **30 minutes** at +**37 °C**.	In manual processing, the microtitre plate is carefully covered tightly with unused cover film.
6	<u>Washing</u> (see 8.4b and 8.4c).	Carry out the washing steps **four times** in total.
7	<u>Substrate reaction</u> Pipette **100 µl** of ready-to-use substrate solution into each well and incubate for **30 minutes** at **room temperature**. The time is calculated from pipetting into the first well.	Masking of the plate is <u>not</u> required. Protect against direct exposure to sunlight.
8	<u>Stopping the reaction</u> Pipette **100 µl** of ready-to-use stop solution <u>into</u> each well.	The substrate solution is not to be removed before adding the stop solution! The same pipetting scheme is to be followed as for the substrate solution.
9	<u>Measurement of the extinction values</u> The extinction values of the single wells are measured in a microtitre plate photometer at 450 nm and the reference wave length 650 nm (620 to 650 nm permitted).	Zero adjustment is done against air. The measurement must be made within 60 minutes of stopping the reaction.
Caution! **Incubation solutions may not flow into other wells. Splashing must be avoided especially when removing and placing the covering film.**		

9 Results

9.1 Evaluation

Cutoff (limit) = the average is formed from the extinction values of both cutoff controls (at the beginning and at the end of the series).

9.1.1 *Qualitative evaluation*

Grey range	lower range = cutoff upper range = cutoff + 20% (cutoff x 1.2)	
Negative	Samples with extinction values **below** the grey range	
Borderline	Samples with extinction values **within** the grey range	
Positive	Samples with extinction values **above** the grey range	

9.1.2 *Quantitative evaluation*

The corresponding antibody activity in **units per ml** is assigned to the extinction values using a formula. The measurement units U/ml are arbitrary units, which do not allow conclusions concerning (interna-tional) reference values.

U/ml sample	(Extinction sample / extinction cutoff) x 20
Grey range	lower range = 20 U/ml upper range = 24 U/ml
Negative	U/ml sample < 20
Borderline	20 ≤ U/ml sample ≤ 24
Positive	U/ml sample > 24

Samples with a borderline test result should be retested. If the results are still borderline after the second test, a further sample should be taken and tested after some time.

The linearity of the test was determined during the evaluation within the following range:
20 U/ml to 105 U/ml (R^2 = 0.99)

In case of an extinction ≥ 3.0 or a measuring value above the linear range, the result should either be given as > 105 U/ml, or the sample may be diluted and measured again. We recommend to start with a final sample dilution of 1:500 and if necessary further subsequent dilution steps.

9.2 Validation - Quality Control

The test can be evaluated under the following conditions:

- The single extinction values of the double analysis of the cutoff control do not deviate by more than 20 % from their average.
- Negative control extinction value ≤ 0.150
- Cut-off control extinction value - Negative control extinction value ≥ 0.050
  (E_{Cutoff} - $E_{neg.\ contr.}$ ≥ 0.050)
- Positive control extinction value - Cutoff control extinction value ≥ 0.300
  ($E_{pos.\ contr.}$ - E_{Cutoff} ≥ 0.300)

These checks are used to validate the test results as per the "Validation Quality Control" chapter. The reproducibility of results can be improved by determining the specific antibodies relative to the cut-off check in U/ml, as the fluctuations from the performance of the test are also included. In validating the test, the positive and negative checks do not need to be evaluated. If necessary, however, they can be carried out for internal quality control purposes. In this case, the results should lie within the target value range given in the analysis certificate or on the label.

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 result does not exclude the possibility of a Chlamydia trachomatis infection. Where there is a clinical suspicion of a Chlamydia trachomatis infection and a negative serological test result, a further sample should be taken and tested 2 weeks later.
- False negative results may occur when the serum samples are taken very soon after infection has occurred.
- A positive recomWell Chlamydia trachomatis test result does not necessarily mean that there is an active illness event.
- Diagnosing a Chlamydia trachomatis infection necessitates the use of the clinical picture and medical history where applicable, in addition to the laboratory measuring values.
- The results of the IgG and IgA detection should be considered as a whole for the assessment of the Chlamydia immune status.
- We generally recommend checking positive and borderline ELISA results by conducting a confirmation test.

11 Test performance

11.1 Diagnostic sensitivity and specificity (Chlamydia trachomatis DNA-positive cervical smear samples)

	*recom*Well Chlamydia trachomatis IgG % (n)	MIF test IgG[1] % (n)	*recom*Well Chlamydia trachomatis IgA % (n)	MIF test IgA[1] % (n)
Diagnostic sensitivity[2]	74.7 (56/75)	78.7 (59/75)	43.4 (33/76)	13.2 (10/76)
Diagnostic specificity[3]	91.9 (68/74)	93.2 (69/74)	98.6 (72/73)	100.0 (73/73)

[1]In-house (external laboratory) micro-immunofluorescence (MIF) test with elementary bodies (EB) of *Chlamydia trachomatis* made by Serovare D-K. Lipopolysaccharide (LPS) was removed. Samples with a titre ≥1:32 were positively integrated into the study.
[2]DNA-positive samples (cervical smear). Samples with a borderline serological result were not incorporated into the study.
[3]DNA-negative samples (cervical smear). Borderline serological results were not incorporated into the study.

11.2 Relative correlation

The determination of positive and negative correlation was carried out in a comparison with a commercially available strip test.

	*recom*Well Chlamydia trachomatis IgG % (n)	*recom*Well Chlamydia trachomatis IgA % (n)
Positive correlation[1]	95.8% (161/168)	90.9% (90/99)
Negative correlation[1]	97.4% (300/308)	97.8% (352/360)

[1]Samples with a borderline serological result were not incorporated into the study.

11.3 Sero-prevalence

	*recom*Well Chlamydia trachomatis IgG		*recom*Well Chlamydia trachomatis IgA	
	Seropositive % (n)	Seronegative % (n)	Seropositive % (n)	Seronegative % (n)
Sero-prevalence among blood donors[1]	11.6% (28/241)	88.4% (213/242)	5.3% (13/244)	94.7% (231/244)

[1]The sero-prevalence for *Chlamydia trachomatis* among the blood donors examined was determined with the aid of a commercially available strip test for IgG (12.0%) and IgA (6.6%). Origin of samples: Bavarian Red Cross. Borderline results were not incorporated into the study.

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 or cross-reactions with potentially interfering antibodies.
a) Interferences: Control studies on potential interfering factors have shown that the performance of the test is not affected by anticoagulants (sodium citrate, EDTA, heparin, CPD), haemolysis, lipaemia or bilirubinaemia of the sample.
b) Cross-reactions: Potential interference from antibodies against EBV, as well as from other conditions that are due to atypical behaviour of the immune system (anti-nuclear auto-antibodies, rheumatoid factor) can be practically ruled out.

11.5 Precision

	*recom*Well Chlamydia trachomatis
Intra-assay variance*	VC(IgG) = 6.8%, VC (IgA) = 5.2%
Inter-assay variance**	VC(IgG) < 7% (6 positive, 2 borderline samples) or < 11% (4 negative samples). VC(IgA) < 9% (5 positive, 2 borderline samples) or < 10% (5 negative samples).

*A patient sample was measured in 96 wells of a micro-titration plate. The variation coefficient (VC) for the extinct values was calculated.
** The recomWell Chlamydia trachomatis test was carried out in 8 separate preparations, using 12 serums with different extinction values.

12 Literature

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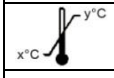
We will gladly send you further literature on the diagnosis of Chlamydia on request.

13 Explanation of symbols

Σ	Content is sufficient for <n> applications Number of applications
WASHBUF 10 X	Wash Buffer (ten times concentration)
DILUBUF	Dilution Buffer
SUBS TMB	Chromogenic substrate Tetramethylbenzidin
SOLN STOP	Stop solution
TAPE	Covering films
MTP	Microtitre plate
CONTROL + IgG	Positive controls IgG
CONTROL ± IgG	Cut-off controls IgG
CONTROL - IgG	Negative controls IgG
CONJ IgG	Anti-human IgG conjugate
CONTROL + IgA	Positive controls IgA
CONTROL ± IgA	Cut-off controls IgA
CONTROL - IgA	Negative controls IgA
CONJ IgA	Anti-human IgA conjugate
TVALUE	Target and / or target range in U / ml
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*Well Chlamydia trachomatis IgG** ***recom*Well Chlamydia trachomatis IgA**	Item no. **6904** Item no. **6905**
Instructions for use valid from	GARECT006EN 2023-03

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