Chlamydien-IgM-rELISA medac

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Chlamydien-IgM-rELISA medac

Recombinant enzyme immunoassay for the qualitative detection of specific IgM antibodies to chlamydial LPS in serum and EDTA-plasma

Cat. no.: 485-TMB

FOR IN VITRO DIAGNOSTIC USE ONLY

Chlamydiae are gram-negative bacterial pathogens. They have an obligate intracellular life cycle in mucosal surfaces, endothelial cells, smooth muscle cells, and according to recent findings in certain tissue structures of the central nervous system. Chlamydiae depend on energy-rich phosphates of their host cells and are therefore energy parasites.

The genus chlamydia comprises four species: ***C. pneumoniae, C. trachomatis, C. psittaci,*** and ***C. pecorum***. ***C. pneumoniae*** and ***C. trachomatis*** are obligate pathogens of humans. ***C. psittaci*** is pathogenic for humans and a variety of animal species. To date, ***C. pecorum*** has been isolated from animals only.

Chlamydia trachomatis is one of the most frequent sexually transmitted pathogens worldwide. It causes infections of the urogenital tract and the eye. In most cases ***C. trachomatis*** infections are asymptomatic. This results in a variety of chronic diseases, which are sustained by the ascended and persisting agents. The clinical pictures include in women: Endometritis, adnexitis, periappendicitis, perihepatitis, and reactive arthritis. As a consequence of repeated adnexitis the tubes occlude which results in sterility.
In men the pathogens may ascend to the epididymis (→ epididymitis) and into the prostate gland (→ prostatitis) after incomplete or unsuccessful treatment of urethritis. A reduction of fertility has been discussed in these cases. Furthermore, post-urethritic reactive arthritis is known in men.
In newborns ***C. trachomatis*** can be transferred during delivery from the infected birth channel to the infant and can cause neonatal conjunctivitis and/or pneumonia.

Chlamydia pneumoniae infections occur worldwide. In addition to flu-like illness, the clinical picture includes sinusitis, pharyngitis, bronchitis, chronic obstructive pulmonary disease, atypical pneumonia, and reactive arthritis. A causal involvement in infection-conditioned asthma, sarcoidosis, atherosclerosis, acute myocardial infarction, stroke, multiple sclerosis, and late-onset of Alzheimer's disease has been discussed.

Chlamydia psittaci infects birds and mammals, which may transmit the pathogen to humans. The resultant frequently severe pneumonias may lead to life-threatening conditions if no targetted antibiotic intervention is started rapidly.

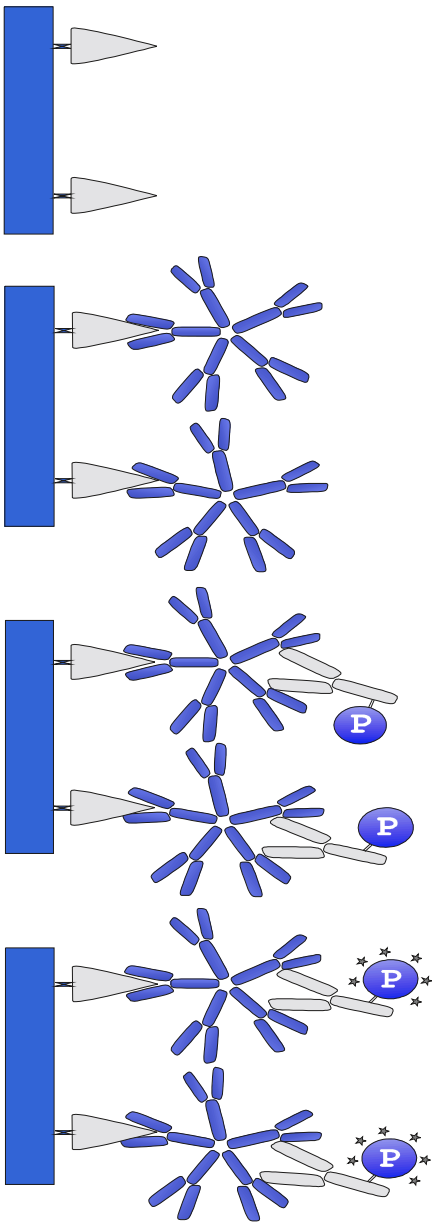
The usual laboratory methods for the detection of chlamydia infections include antigen and antibody determinations. The antigen detection (IFA, ELISA, PCR) is of value for the diagnosis of peripherally localized infections. In ascended courses of disease the detection of the pathogen is limited and has to be complemented by serology. Serological methods include complement fixation test, IFA, MIF, genus- and species-specific ELISA systems.

Chlamydiae contain as common immunodominant antigen the lipopolysaccharide (LPS), to which the first immune reaction is directed. The corresponding antibodies are already detectable within a few days after infection and, thus, allow early diagnosis. The use of paired samples enables the discrimination of current infections, reinfections, and reactivations (defined titer increases) from chronic persistent ones (constant antibody titers). Because of the limited persistence of the LPS antibodies after successful eradication of the pathogens, the diagnosis of current infections is obviously not influenced by past infections.

The Chlamydien-IgG-, IgA-, and IgM-rELISA medac are based upon a molecularly defined, genetechnologically produced antigen. It is an exclusively chlamydia-specific fragment from the LPS which has not been found in any other bacterial LPS. The comparison of sera from blood donors with sera from patients with urogenital or respiratory infections has shown, that antibodies to chlamydial LPS are detected more frequently in patients than in blood donors.

As the reaction is genus-specific, the test result will not differentiate betweeen the chlamydia species. However, as the clinical picture already will hint to the suspected chlamydia species and additionally all the chlamydiae show an equal sensitivity towards specific antibiotics, the genus-specific reactivity does not hamper the consequences.

TEST PRINCIPLE



The plate is coated with chlamydia-specific recombinant LPS fragment.

The chlamydia-specific LPS antibodies from the specimen bind to the antigen.

Peroxidase-conjugated anti-human IgM antibodies bind to the IgM antibodies (P = peroxidase).

Incubation with TMB-substrate (*). The reaction is stopped by the addition of sulfuric acid. The absorption is read photometrically.

Advantages of the test

- ☞ Chemically defined, chlamydia-specific antigen.
- ☞ The breakable microwell strips permit efficient use of the test.
- ☞ Suitable for automation on open ELISA devices.

KIT CONTENTS
Cat. no.: 485-TMB

1. **MTP**
   Microplate: 12 x 8 wells (printed with **CHM**, with frame and desiccant vacuum sealed in aluminium bag), breakable, U-form, coated with chlamydia-specific, recombinant LPS fragment and NBCS, ready to use.

2. **CONTROL | –**
   Negative control: 1 vial with 1.5 ml, human serum, ready to use, stained blue, contains NBCS, phenol (0.09 %), ProClin™ 300 (< 0.0015 % CMIT/MIT)[1,2] and gentamycin sulfate (0.005 %).

3. **CONTROL | +**
   Positive control: 1 vial with 1.5 ml, human serum, ready to use, stained blue, contains BSA, phenol (0.09 %), ProClin™ 300 (< 0.0015 % CMIT/MIT)[1,2] and gentamycin sulfate (0.005 %).

4. **WB**
   Wash buffer: 1 bottle with 100 ml, 0.1 M PBS/Tween (10x), pH 7.2 – 7.4, contains ProClin™ 300 (0.017 % CMIT/MIT)[1].

5. **BAC-DIL**
   Sample diluent: 1 bottle with 110 ml, 0.01 M PBS/Tween/NBCS, pH 7.0 – 7.2, ready to use, stained blue, contains ProClin™ 300 (0.008 % CMIT/MIT)[1].

6. **CON**
   Conjugate: 3 vials with 4.5 ml each, goat anti-human IgM, HRP-conjugated, ready to use, stained red, contains BSA, phenol (0.09 %), ProClin™ 300 (< 0.0015 % CMIT/MIT)[1,2] and gentamycin sulfate (0.005 %).

7. **TMB**
   TMB-substrate: 1 vial with 10 ml, ready to use.

8. **STOP**
   Stop solution: 2 vials with 11 ml each, 0.5 M sulfuric acid (H_2SO_4), ready to use.
   May be corrosive to metals.

9. **RF-ABS**
   IgG/Rf-absorbent: 1 vial with 4 ml, goat anti-human IgG antiserum, ready to use, contains sodium azide (0.025 %).

[1] Mixture of: 5-Chloro-2-methyl-2H-isothiazol-3-one and 2-Methyl-2H-isothiazol-3-one (3:1).
[2] May produce an allergic reaction. Safety data sheet available on request (see CD-ROM).

1. STORAGE AND STABILITY

Material/Reagent	State	Storage	Stability
Test kit	unopened	2...8 °C	until expiry date
Microplate	opened	2...8 °C in bag with des-iccant	6 weeks
Controls	opened	2...8 °C	6 weeks
Wash buffer	diluted	2...8 °C	6 weeks
Sample diluent	opened	2...8 °C	6 weeks
Conjugate	opened	2...8 °C	6 weeks
TMB-substrate	opened	2...8 °C	6 weeks
Stop solution	opened	2...8 °C	until expiry date
IgG/Rf-absorbent	opened	2...8 °C	6 weeks

Do not use the reagents after the expiry date.

2. REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED

2.1. Distilled or deionised water.

2.2. Adjustable micropipettes.

2.3. Clean glass or plastic containers for dilution of wash buffer and specimen.

2.4. Suitable device for microplate washing (e.g. multistepper or ELISA washer).

2.5. Incubator for 37 °C.

2.6. Microplate reader with filters for 450 nm and 620 – 650 nm.

3. PREPARATION OF THE REAGENTS

Before starting the test procedure all kit components must be equilibrated to room temperature (RT).

Calculate the number of wells required.

3.1. Microplate
After each removal of wells the aluminium bag has to be tightly resealed together with the desiccant. Storage and stability of the wells are indicated under point 1.

3.2. Wash buffer
Mix one volume of wash buffer (10x) with nine volumes of distilled/deionised water (e.g. 50 ml wash buffer (10x) with 450 ml water). 10 ml of diluted wash buffer are needed for eight wells.

Crystals in the wash buffer (10x) have to be dissolved by warming (max. 37 °C) and/or stirring at RT.

Do not mix test specific reagents (microplate, controls, conjugate) from different kit lots. In contrast to that, sample diluent, wash buffer, TMB-substrate, IgG/Rf-absorbent and stop solution are generally exchangeable in all Chlamydia and Mycoplasma-ELISA medac.

Reagents from other manufacturers must not be used in general.

Valid and reproducible results are only obtained if the test procedure is precisely followed.

4. SPECIMEN

4.1. The test is suitable for serum samples and EDTA-plasma. The patient samples can be stored for 7 days at 2-8 °C. Long term storage should be performed at ≤ -20 °C. Repeated thawing and freezing of the samples has to be avoided.

4.2. Pretreatment of the samples, e.g. inactivation, is not necessary. However, they should neither be contaminated with microorganisms nor contain red blood cells.

4.3. The samples are employed at a final dilution of 1:50 after IgG/Rf absorption (see 5.A.).

5. TEST PROCEDURE

5.A. IgG/RF ABSORPTION

Attention:

* **The controls are ready to use (no absorption necessary).**
* **The volumes indicated in the following are for single determinations only.**

5.A.1. Sample: 10 µl of the sample are diluted with 240 µl sample diluent (dilution 1:25).

5.A.2. Absorption: 30 µl IgG/Rf-absorbent and 30 µl diluted sample are mixed (dilution 1:50) and incubated for 15 min at RT.

Alternative: The absorption can be performed overnight at 2 – 8 °C.

5.A.3 The test dilution is now 1:50.

5.B. TEST PROCEDURE

5.B.1. Cut the aluminium bag above the zip fastener and take out the required number of microplate wells (see 3.1.).

Microplate wells are ready to use and do not have to be pre-washed.

5.B.2. Pipette 50 µl of sample diluent into the well A1 as blank (see 6.A.), 50 µl of the negative control in duplicate, and each 50 µl of the positive control and the diluted patient samples for single determinations.

If necessary, the microplate wells can be kept in a humid chamber up to 30 min at 2 – 8 °C before proceeding.

5.B.3. Incubate the microplate wells for 60 min (± 5 min) at 37 °C (± 1 °C) in a humid chamber or sealed with incubation cover foil.

5.B.4. After incubation wash the microplate wells three times with each 200 µl wash buffer per well. Pay attention that all wells are filled. After washing tap microplate wells on filter paper.

Do not allow the wells to dry out! Proceed immediately!

5.B.5. Add conjugate (coloured red) to each well.

50 µl of conjugate have to be pipetted into the wells if the test is done manually.

Please note:
When working with automated instruments, 60 µl of conjugate have to be pipetted into each well due to a higher evaporation in the incubation chambers of the automates.

The principal suitability of the test for automation could be shown during validation. Nevertheless we recommend to verify the compatibility with the automate employed.

5.B.6. Incubate the microplate wells again for 60 min (± 5 min) at 37 °C (± 1 °C) in a humid chamber or sealed with incubation cover foil.

5.B.7. After incubation wash microplate wells again (see 5.B.4.).

5.B.8. Add 50 µl of TMB-substrate to each well and incubate the microplate wells for 30 min (± 2 min) at 37 °C (± 1 °C) in a humid chamber or sealed with incubation cover foil in the dark. Positive samples turn blue.

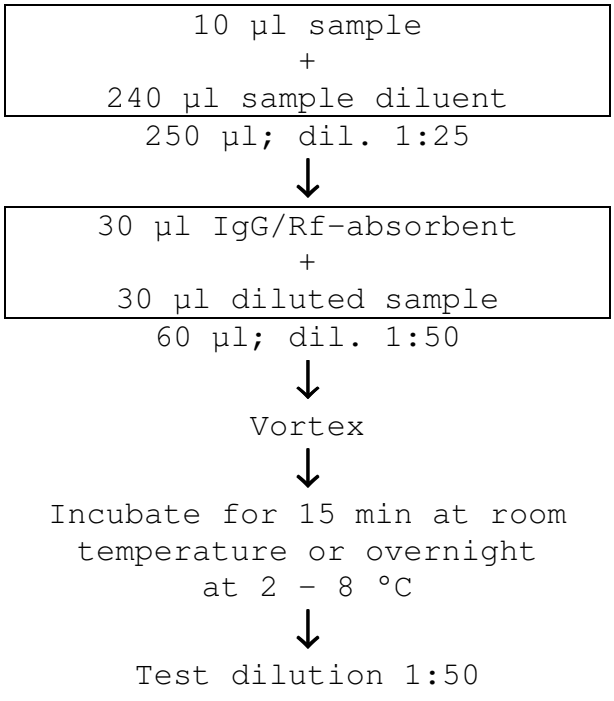
5.B.9. Stop the reaction by adding 100 µl of stop solution to each well. Positive samples turn yellow.

Clean microplate wells from underneath before the photometric reading and take care that there are no air bubbles in the wells.

The reading should be done within 15 min after adding the stop solution.

5.C. TABLE FOR THE IgG/Rf-ABSORPTION

Indication per determination



5.D. TABLE FOR THE TEST PROCEDURE

	Blank (A1)	Negative control	Positive control	Sample
Sample Diluent	50 µl	–	–	–
Negative control	–	50 µl	–	–
Positive control	–	–	50 µl	–
Sample	–	–	–	50 µl
Incubate for 60 min at 37 °C, wash 3 x with 200 µl wash buffer				
Conjugate	50/60 µl*)	50/60 µl*)	50/60 µl*)	50/60 µl*)
Incubate for 60 min at 37 °C, wash 3 x with 200 µl wash buffer				
TMB-substrate	50 µl	50 µl	50 µl	50 µl
Incubate for 30 min at 37 °C in the dark				
Stop solution	100 µl	100 µl	100 µl	100 µl
Photometric reading at 450 nm (ref. 620 – 650 nm)				

*) manual/automatic procedure (see 5.B.5.)

6.A. CALCULATION OF RESULTS (VALIDITY)

* Read OD values at 450 nm (reference wavelength 620 – 650 nm).

* Subtract the OD value of the blank (well A1) from all other OD values.

* The OD value of the blank has to be **< 0.100.**

* The mean OD value of the **negative control** has to be **< 0.200.**

* The OD value of the **positive control** has to be **> 0.800.**

* **Cut-off = Mean OD value of the negative control + 0.370**

* **Grey zone = Cut-off ± 15 %**

Repeat the run if the results do not meet the specification.

6.B. EVALUATION OF RESULTS

6.B.1.QUALITATIVE

Result	Valuation
OD < Grey zone	negative
OD of Cut-off ± 15 %	equivocal
OD > Grey zone	positive

6.B.2.QUANTITATIVE

With the Chlamydien-IgM-rELISA medac the samples have to be titrated if endtiter determination is desired.

6.C. INTERPRETATION OF THE RESULTS

6.C.1.CRITERIA FOR SIGNIFICANT TITER INCREASES

For the diagnosis of current, fresh infections, reinfections, reactivations (significant titer increases) and discrimination from persistent infections (constant antibody titers) we principally recommend the use of paired samples. They should be obtained 10 - 14 days apart.

The following criteria have been described as a possibility for the assessment of significant titer increases (Persson et al., Verkooyen et al.):

Threefold or greater increase in
specific IgG **or** IgA antibody titers
or
twofold or greater increase in
specific IgG **and** IgA antibody titers
or
twofold or greater increase in
specific IgM antibody titers

6.C.2. SPECIFIC IgM INTERPRETATION

Possible Results	Interpretation
+	Indication of acute infection.
+/-	Possibility of a very early stage of acute or recent infection. Retest IgM after 10 - 14 days and test for IgA and IgG.
-	In cases of justified clinical suspicion test for IgA and IgG.

* The IgM results should always be interpreted in connection with IgG and/or IgA, the clinical picture and additional diagnostic parameters.

* Samples with OD values within the grey zone should be retested together with a fresh specimen taken 14 days later in order to determine a titer change.

* High concentrations of hemoglobin and of bilirubin do not have an influence on the results.

* In rare cases, high lipid concentrations may influence the test results.

* Cross-reactivities with antibodies to parvovirus B19 cannot be excluded in individual cases.

Comment:
In cases of fresh acute chlamydial infections the serological antibody results may be negative despite clinics and positive antigen detection. If a serological confirmation of a positive antigen result or if a follow-up is desired we recommend to test after 10 - 14 days for seroconversion.

7. PERFORMANCE CHARACTERISTICS

We determined the following performance characteristics during the diagnostic evaluation.

7.A. PREVALENCE

Sera from various patients cohorts (culture-positive, -negative STD patients, prostitutes, infertile females, infertile males, patients with rheumatic diseases, patients with chronic obstructive respiratory diseases [COPD]), and controls (pregnant women, 2 blood donor cohorts) were investigated for the **determination of LPS antibody prevalence.**

Cohorts	**Prevalence**		
	IgG	**IgA**	**IgM**
Culture-positive STD patients (medac-internal investigations)	**67 % (88/132)**	**56 % (74/132)**	**14 % (18/132)**
Culture-negative STD patients (medac-internal investigations)	**33 % (41/125)**	**13 % (16/125)**	**2 % (3/125)**
Prostitutes (Schmitz et al.)	**86 % (255/295)**	**48 % (141/295)**	**21 % (63/295)**
Infertile females (Schmitz et al.)	**75 % (62/83)**	**45 % (37/83)**	**10 % (8/83)**
Infertile males (Schmitz et al.)	**71 % (144/203)**	**35 % (71/203)**	**9 % (18/203)**
Patients with rheumatic diseases (medac-internal investigations)	**83 % (158/191)**	**63 % (120/191)**	**7 % (13/191)**
COPD patients (Verkooyen et al. 1997)	**53 % (144/271)**	**32 % (88/271)**	**3 % (9/271)**
Pregnant women (medac-internal investigations)	**32 % (62/192)**	**17 % (32/192)**	**8 % (16/192)**
Blood donor group 1 (Verkooyen et al. 1998)	**29 % (325/1104)**	**n.d***	**n.d***
Blood donor group 2 (medac-internal investigations)	**37 % (154/416)**	**13 % (54/416)**	**3 % (6/240)**

*: n.d = not determined

7.B. PRECISION

Sample	Intra-assay variation				Sample	Inter-assay variation (n = 11)		
	mean OD	SD	CV (%)	n		mean OD	SD	CV (%)
NC	0.046	0.003	6	24	**NC**	0.041	0.003	7
BC	0.639	0.017	3	24	**BC**	0.601	0.026	4
PC	1.534	0.040	3	24	**PC**	1.439	0.059	4
N° 1	0.446	0.018	4	24	**N° 3**	0.421	0.030	7
N° 2	0.986	0.030	3	24	**N° 4**	1.005	0.055	5

NC = negative control; BC = weak positive control (not included in the kit); PC = positive control

GENERAL HANDLING ADVICES

* Do not exchange the vials and their screw caps in order to avoid cross contamination.

* The reagents have to be sealed immediately after use in oder to avoid evaporation and microbial contamination.

* After use, the reagents have to be stored as indicated to guarantee the shelf life.

* After use, all components of the testkit should be stored in the orginal package, in order to avoid mixing up the reagents of other test systems or lots (see also 3.).

HEALTH AND SAFETY INFORMATION

* The local occupational safety and health regulations have to be regarded.

* Reagents of human origin have been tested and found to be negative for HBsAg, for antibodies to HIV-1/2 and to HCV. Nevertheless, it is strongly recommended that these materials as well as those of animal origin (see kit contents), should be handled as potentially infectious and used with all necessary precautions.

DISPOSAL CONSIDERATIONS

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

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