Chlamydia pneumoniae-IgG-ELISA *plus* medac

English

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Chlamydia pneumoniae-IgG-ELISA *plus* medac

Enzyme immunoassay for the quantitative detection of IgG antibodies to ***Chlamydia pneumoniae*** in serum and plasma

Cat.no.: 430-PLUS

FOR IN VITRO DIAGNOSTIC USE ONLY

INTRODUCTION

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. trachomatis***, ***C. pneumoniae***, ***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.

Infections with ***C. pneumoniae*** occur worldwide. The spectrum of diseases, in addition to flu-like illness, includes sinusitis, pharyngitis, bronchitis, chronic obstructive pulmonary diseases, pneumonia, and reactive arthritis. A causal involvement of ***C. pneumoniae*** in infectious asthma, sarcoidosis, lung cancer, atherosclerosis, acute myocardial infarction, brain stroke, multiple sclerosis, and late onset of Alzheimer's disease remains an area of actual investigation.

According to Grayston and Saikku (1989), who firstly had described this chlamydia species, almost everybody is infected and reinfected with ***C. pneumoniae*** throughout his life. The mainly weak and/or diffuse symptomatic course of ***C. pneumoniae*** infections makes their detection more difficult; undetected infections may lead to chronic courses of disease with serious sequelae.

The diagnosis of ***C. pneumoniae*** infections is based upon isolation of the pathogen from cell culture, direct antigen detection, nucleic acid amplification tests, and serology. Isolation of the pathogen from cell culture normally needs various subsequent passages; it is time consuming, restricted to special laboratories, and only in few cases successful. Direct immunofluorescence assays (IFA) and antigen enzyme immunoassays (EIA) have not been widely used; they have been reported

to be of low sensitivity and also specificity. Until now a commercial, standardized molecular biological detection method as PCR has not become accepted.

The disadavantages of culture and antigen detection for diagnosis of ***C. pneumoniae*** infection have made serology the method of choice.

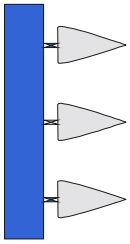
For the detection of species-specific antibodies the microimmunofluorescence (MIF) has been considered the "gold standard". MIF is laborious, subjective, and requires much experience. This test system is not standardized; the use of different antigens and different cut-off criteria for past and recent or current infections creates significant lab-to-lab variations of results.

The ELISA technique is the favoured method in laboratory routine at this time. The quality of the diagnostic information depends as well on the employed antigen as on the method of diagnostic data interpretation. The **Chlamydia pneumoniae-IgG-ELISA *plus* medac** employs a highly purified and specific antigen which is also used in **Chlamydia pneumoniae-IgA-ELISA *plus* medac** and **Chlamydia pneumoniae-IgM-sELISA medac**.

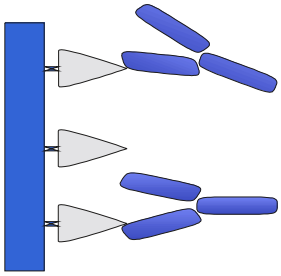
The detection of IgM, IgA and IgG antibodies to ***C. pneumoniae*** in various combination allows a classification of the state of infection.

Moreover, the medac one-point-quantification (AU/ml) provides the best preconditions for reproducible results and in this way for measuring of the antibody courses.

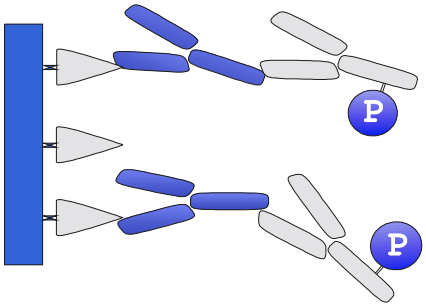
TEST PRINCIPLE



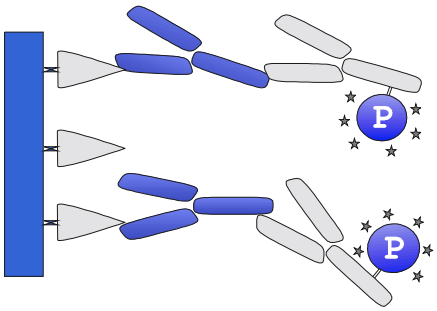
The plate is coated with a highly purified ***C. pneumoniae***-specific antigen preparation.



The ***C. pneumoniae***-specific antibodies from the specimen bind to the antigen.



Peroxidase-conjugated anti-human IgG antibodies bind to the IgG 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

- ☞ High sensitivity and specificity.
- ☞ One-point-quantification, no standard curve needed.
- ☞ The breakable microwell strips permit efficient use of the test.

KIT CONTENTS
Cat. no.: 430-PLUS

1. **MTP**
   Microplate: 12 x 8 wells, (printed with **QPG**, with frame and desiccant vacuum sealed in aluminium bag), breakable, U-form, coated with ***C. pneumoniae***-specific antigen and FCS, ready to use.

2. **CONTROL** | **-**
   Negative control: 1 vial with 1.5 ml, human serum, ready to use, stained blue, contains NBCS, phenol, ProClin™ 300 and gentamycin sulfate.

3. **CONTROL** | **+**
   Positive control: 1 vial with 1.5 ml, human serum, ready to use, stained blue, contains BSA, FCS, phenol, ProClin™ 300 and gentamycin sulfate.

4. **CAL**
   Calibrator: 1 vial with 1.5 ml, human serum, ready to use, stained blue, contains BSA, FCS, phenol, ProClin™ 300 and gentamycin sulfate.

5. **WB**
   Wash buffer: 1 bottle with 100 ml PBS/Tween (10x), pH 7.2 – 7.4, contains ProClin™ 300.

6. **BAC-DIL**
   Sample diluent: 1 bottle with 110 ml PBS/Tween/NBCS, pH 7.0 – 7.2, ready to use, stained blue, contains ProClin™ 300.

7. **CON**
   Conjugate: 3 vials with 4.5 ml each, goat anti-human IgG, HRP-conjugated, ready to use, stained green, contains BSA, phenol, ProClin™ 300 and gentamycin sulfate.

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

9. **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.

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 desiccant	12 weeks
Controls/Calibrator	opened	2...8 °C	12 weeks
Wash buffer	diluted	2...8 °C	12 weeks
Sample diluent	opened	2...8 °C	12 weeks
Conjugate	opened	2...8 °C	12 weeks
TMB-substrate	opened	2...8 °C	12 weeks
Stop solution	opened	2...8 °C	until expiry date

Do not use the reagents after the expiry date.

2. REAGENTS AND MATERIALS REQUIRED BUT NOT SUPPLIED

2.1. Distilled or deionised water.

2.1. Adjustable micropipettes.

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

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

2.4. Incubator for 37 °C.

2.5. 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, calibrator, conjugate) from different kit lots. In contrast to that, sample diluent, wash buffer, TMB-substrate 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 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 any red blood cells.

4.3. The samples have to be diluted 1:250 with sample diluent. We recommend to prepare an initial dilution of 1:50 (e. g 10 µl sample + 490 µl sample diluent) and then a further dilution of 1:5 (e.g. 10 µl 1:50 prediluted sample + 40 µl sample diluent). Samples beyond the measuring range can be further diluted.

5.A. TEST PROCEDURE

5.1. 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.

5.2. Pipette 50 µl sample diluent into well A1 as blank (see 6.A.). Add 50 µl each of the negative control, the positive control as well as the diluted samples in single determination, and 50 µl of the calibrator in duplicate to the wells.

If necessary the microplate wells can be kept up to 30 min at RT before proceeding.

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

5.4. After incubation wash the microplate wells three times with 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!

5.5. Add conjugate (coloured green) 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 devices, 60 µl of conjugate have to be pipetted into each well due to a higher evaporation in the incubation chambers of the devices.

The suitability of the test for automated devices was confirmed during the evaluation of the test. Nevertheless we recommend to verify the compatibility of the test with the devices used in the lab.

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

5.7. After incubation wash microplate wells again (see 5.4.).

5.8. Add 50 µl of TMB-substrate to each well and incubate 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.

5.9. 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 must be done within 15 min after adding the stop solution.

5.B. TABLE FOR THE TEST PROCEDURE

	Blank (A1)	Negative control	Positive control	Calibrator	Sample
Sample diluent	50 µl	–	–	–	–
Neg. control	–	50 µl	–	–	–
Pos. control	–	–	50 µl	–	–
Calibrator	–	–	–	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*)	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	50 µl
Incubate for 30 min at 37 °C in the dark					
Stop solution	100 µl	100 µl	100 µl	100 µl	100 µl
Photometric reading at 450 nm (ref. 620 – 650 nm)					

*) manual/automatic procedure (see 5.5.)

6.A. CALCULATION OF RESULTS (VALIDITY)

* The evaluation is performed using arbitrary units (AU/ml).

* 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.

* Lot-specific data

  The lot-specific data sheet provided with the kit contains the following information:
  - Lot-specific calibration curve
  - Curve parameters a and b
  - Nominal OD value of the calibrator
  - Lower OD limit of the calibrator
  - Nominal concentration range (AU/ml) of the positive control

* Validity criteria

- The OD value of the **blank** has to be **< 0.100.**
- The OD value of the **negative control** has to be **< 0.100.**
- The unit value of the **positive control** has to be within the nominal range indicated in the lot-specific data sheet.
- The mean OD value of the **calibrator** has to be above the lower OD limit indicated in the lot-specific data sheet.

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

* Correction of the results

The measured OD values of the positive control and the samples have to be corrected as follows:

$$OD_{corrected} = \frac{\text{Nominal OD value of the calibrator}}{\text{Measured OD value of the calibrator}} \times OD_{measured}$$

* Quantification of the results

The corresponding concentrations of the corrected OD values in AU/ml can be read from the lot-specific calibration curve (see lot-specific data sheet).

Alternatively, the concentrations can be calculated using the following formula:

$$\text{Concentration [AU/ml]} = b/\left(\frac{a}{OD_{corrected}} - 1\right)$$

Most of the new ELISA readers allow to program the formula, thus enabling an automated data processing.

The measuring range spans from 22 to 500 AU/ml. Samples below this range have to be interpreted as < 22 AU/ml, those above as > 500 AU/ml. These values must not be extrapolated.

The cut-off is 25 AU/ml.

Grey zone = 22 - 28 AU/ml.

Attention! Important note!

Due to the mathematical algorithm of the quantification negative or not defined AU values can be obtained in following cases:

- Highly positive samples with corrected OD-values ≥ a are calculated as negative or not defined AU values (not allowed division by 0). These samples have to be retested in higher dilutions or have to be interpreted as > 500 AU/ml.

6.B. INTERPRETATION OF RESULTS/LIMITATIONS OF THE METHOD

* Samples with Unit values below the lower limit of the grey zone are reported as **NEGATIVE.**

* Samples with Unit values within the grey zone are reported as **EQUIVOCAL.**

  These samples should be retested together with a fresh specimen taken 14 days later in order to determine a titer change.

* Samples with Unit values exceeding the upper limit of the grey zone are reported as **POSITIVE.**

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

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

* Cross-reactivities with antinuclear antibodies, heterophilic antibodies and antibodies to ***C. psittaci*** as well as to ***C. trachomatis*** cannot be excluded in individual cases.

6.C. SPECIFIC IgM-/IgA-/IgG-INTERPRETATION

Possible results IgM COI*	 IgA AU/ml	 IgG AU/ml	Interpretation
>1.1 +	<22 -	<22 -	Serological indication of early stage of infection or polyclonal B-cell stimulation. Retest IgM, IgA and IgG after 14 days.
>1.1 +	>28 +	<22 -	Serological indication of acute infection. Retest IgG after 14 days.
>1.1 +	<22 -	>28 +	Serological indication of acute infection.
>1,1 +	>28 +	>28 +	Serological indication of acute infection.
<0.9 -	>28 +	>28 +	Serological indication of current infection[1]. Retest IgA and IgG after 14 days.
<0.9 -	<22 -	>28 +	Serological indication of past infection. In case of clinical suspicion retest after 14 days for IgA and IgG antibody courses.
<0.9 -	>28 +	<22 -	Serological indication of early stage of infection or solitary, persisting IgA[2]. Retest IgM, IgA and IgG after 14 days.
<0.9 -	<22 -	<22 -	No serological indication of current or past infection[3]. In case of clinical suspicion retest after 14 days for IgM, IgA and IgG antibodies.

*** Cut-off-Index**

Comment:
Borderline results may indicate commencing or subsiding infections. Retesting after 14 days is recommended.

[1] A current infection may stand for:
- chronic infection with persisting pathogens: The unit values of the antibodies remain constant for several weeks.
- acute infection: The unit values of the antibodies increase. A **twofold** increase of the **IgG-unit value** is considered as statistically significant elevated.
  A **two and a half times** increase of the **IgA-unit value** is considered as statistically significant elevated.
- high IgG concentration: An acute infection cannot be excluded. According to Grayston et al. (1989), MIF IgG titres ≥ 1:512 speak for an acute infection.

[2] In individual cases solitary IgA antibodies may persist. This immunological phenomenon appears in different bacteriological infections. A clinical relevance is not appraisable.

[3] In cases of fresh acute ***C. pneumoniae*** infections the serological antibody results may be negative despite clinical symptoms 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 14 days for sero-conversion.

7. PERFORMANCE CHARACTERISTICS

We determined the following performance characteristics during the diagnostic evaluation.

7.A. SPECIFICITY AND SENSITIVITY

In the frame of the diagnostic evaluation 560 samples were measured in comparison to the 1st test generation of the **C. pneumoniae-IgG-ELISA *plus* medac.**

The comparison showed the following results*:

Sensitivity	=	100%
Specificity	=	96.8%
Concordance	=	99.5%

*Borderline results had not been considered.

7.B. PRECISION

Sample	Intra-assay Variation				Sample	Inter-assay Variation (n = 11)		
	∅ AU/ml	SD	CV (%)	n		∅ AU/ml	SD	CV (%)
PC	60.6	2.9	4.8	22	**PC**	74.7	5.8	7.8
1	13.3	0.5	4.1	22	**5**	12.5	1.1	9.2
2	55.3	4.6	8.4	22	**6**	58.5	4.4	7.6
3	141.9	12.5	8.8	22	**7**	119.7	8.9	7.4
4	397.4	39.3	9.9	22	**8**	432.3	28.9	6.7

GENERAL HANDLING ADVICES

* To avoid cross-contamination do not exchange the vials and their screw caps.

* The reagents have to be sealed immediately after use 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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