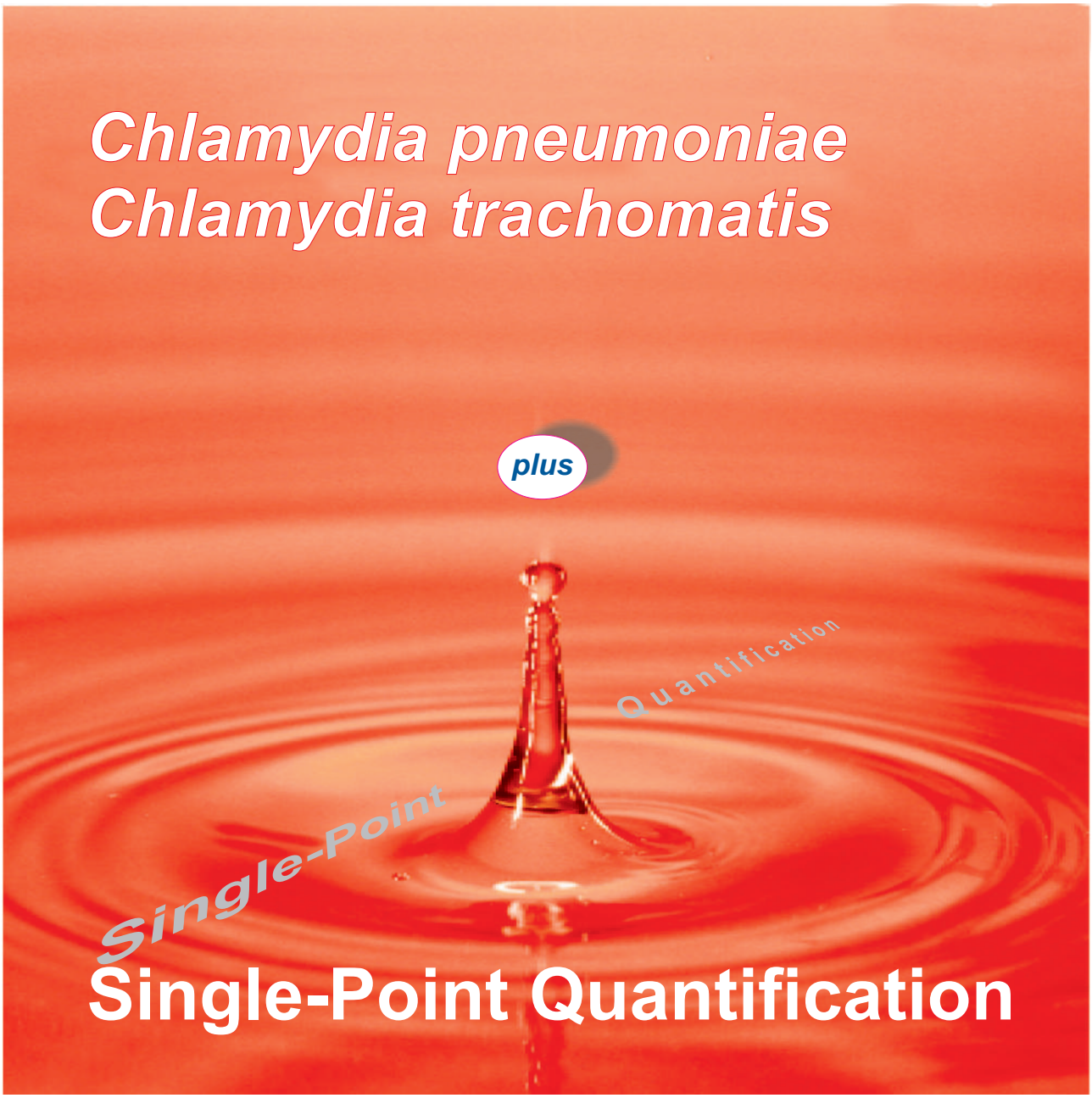
medac
diagnostics



C. pneumoniae IgG and IgA ELISA *plus*

C. trachomatis IgG and IgA ELISA *plus*

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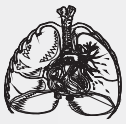
Chlamydia pneumoniae Infections

Chlamydia pneumoniae* is an intracellular parasite which colonises, in a first step, the mucous epithelia of the respiratory tract. The pathogen is distributed by monocytes and may also reach the plaques of blood vessels, the synovia or the central nervous system.
The infection often begins quite uncharacteristically with pharyngitis, cough or a cold in the nose. Confusing this infection with a flu-like illness is not uncommon. The course of the disease can be protracted. Not recognised ***C. pneumoniae*** infections may often lead to severe sequelae. The incidence of acute primary ***C. pneumoniae*** infections reaches its highest level at 9% per year in children between 5 and 9 years of age. Repeated reinfections are responsible for the high seroprevalence of IgG antibodies in adults (50% up to >75%).



Infections of the respiratory tract

C. pneumoniae is the cause of 10-15% of all cases of atypical pneumonia. ***C. pneumoniae*** has also been held responsible for 5% of all infections of the bronchi and nasal sinuses. ***C. pneumoniae*** is also involved in the causation of otitis media and infection-conditioned asthma, though its frequency has not been determined yet.

Chlamydia-induced arthritis (CIA)

CIA, which conventionally is caused by ***C. trachomatis***, has also been reported as sequelae after bronchopulmonary infection with ***C. pneumoniae***. The pathogen was repeatedly detected in the synovia. Vital pathogens in the joints and persisting IgG and IgA antibodies towards the pathogens underline the possible involvement of C. pneumoniae in the genesis of this arthritis form.

C. pneumoniae also seems to play a role in the pathogenesis of vasculitis.

Atherosclerosis

Vital, culturable pathogens in the plaques of blood vessels as well as serological findings indicate an association between infection and development of plaques. Extensive treatment of ***C. pneumoniae***-positive patients with macrolides has not yet proven to protect from the progression of arterial obstructive disease. Further studies are needed to identify clearly the potential role of ***C. pneumoniae*** in the pathogenesis of atherosclerosis. Additionally, C. pneumoniae seems to be also involved in the etiology of myocarditis and endocarditis.

Under consideration

Multiple Sclerosis

Various findings speak for a multifactorial aetiology of multiple sclerosis. The interaction between genetic disposition and triggering environmental agents which may induce an autoimmune event seems probable. An involvement of ***C. pneumoniae*** as an external trigger in the aetiology of multiple sclerosis has been considered. Both pathogens and specific IgG antibodies had already been demonstrated in cerebrospinal fluid.

Alzheimer's disease

Further investigations have to clarify if ***C. pneumoniae*** may also be considered a risk factor responsible for neuropathological changes in late type Alzheimer's disease. In individual cases, material of the pathogen was demonstrated in diseased brain areas. In addition, specific antibodies were detected in the liquor.

* Synonym: *Chlamydophila pneumoniae*

Chlamydia trachomatis Infections

Chlamydia trachomatis is an intracellular parasite which colonises, in an first step, the mucous epithelia of the urogenital tract of men and women. The pathogen may also be distributed by monocytes to the synovia. In western industrialised countries, ***C. trachomatis*** infection is the most frequent sexually transmitted bacterial disease. After predominantly asymptomatic, inapparent primary infections and reinfections, frequently only after years severe sequelae may develop. The highest prevalance of fresh ***C. trachomatis*** infections is 6%-8% between 15 and 25 years of age. ***C. trachomatis***-conditioned diseases are predomonantly sex-specific.

Urethritis, cervicitis, endometritis, adnexitis, perihepatitis, periappendicitis etc.

In women, ***C. trachomatis*** colonises initially the mucous epithelia of the lower genital tract in varying density. In 50% to 60% of all cases both urethra and cervix are infected. ***C. trachomatis*** is predestined to ascend via the mucosa. Thus, finally the pathogen reaches via the cervix, uterus, and tubes the abdominal cavity. En route the pathogen causes repeated inflammations which may lead to the above mentioned clinical manifestations. At this stage in the majority of cases peripheral pathogens are no longer detectable.

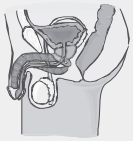
In pregnant women, *C. trachomatis*-conditioned cervicitis

may lead to infection of the newborn during birth. Within less than 4 weeks such infection may result in inclusion conjunctivitis or even manifest as severe newborn pneumonia.

Childlessness

About every 7th marriage is currently affected by involuntary infertility. ***C. trachomatis*** is the main cause of infection-conditioned sterility. More than half of these sterility cases may be related to such infections. In the extreme case ***C. trachomatis*** infections may lead to a complete loss of tubal function. Chronic ***C. trachomatis*** infections increase the risk of ectopic pregnancy 5-fold. Moreover, adverse effects on implantantion rates as well as early abortion may be induced, too.

Non-gonorrheal urethritis, prostatitis, epididymitis



In men, up to 50% of non-gonorrheal urethritis cases are caused by ***C. trachomatis***. First symptoms may appear 1-3 weeks after onset of infection. Because of the often weak and diffuse symptoms the infection is frequently recognised too late. The ascended pathogens may lead to chronic inflammations in the prostate gland and the epididymis. Their impact on male sterility cannot be judged with reasonable certainty. In any case the danger of transmitting the infection to the female partner is assured.

Proctitis

Especially in connection with anal intercourse, the rectum is at risk of getting a ***C. trachomatis*** infection. Due to bacterial pre-damage of the mucosa the invasion of HIV is favoured.

Chlamydia-induced arthritis (CIA)

CIA is a reactive arthritis form which may develop weeks or only months after a ***C. trachomatis***-conditioned non-gonorrheal urethritis, cervicitis or bartholinitis. Except for clinically symptomatic episodes, the latent persisting form of the pathogens dominates. In most of the cases C. trachomatis is no more detectable at the portal of entry.

C. pneumoniae and *C. trachomatis* Diagnosis

Direct detection

Nucleic acid amplification technique

In suspected acute, peripheral ***C. trachomatis*** infections nucleic acid amplification techniques (NAAT) such as PCR are employed more and more frequently. In contrast, in ***C. pneumoniae*** infections such molecular biology techniques are less common.

Immunofluorescence assay/enzyme immunoassay

Furthermore, for direct detection of both chlamydia species immunofluorescence assays (IFA) and enzyme immunoassays (EIA) are used although they are less specific and also less sensitive than the molecular biology techniques.

Serology

Diagnosis of a chlamydia infection may also be performed by detection of chlamydia-specific antibodies. Particularly in progressed chronic chlamydia infections serology is method of choice. In these cases frequently no pathogens are detectable peripherally anymore.

Microimmunofluorescence test

The microimmunofluorescence (MIF) is still considered the Gold Standard. The quality of MIF results suffers from the subjective assessment of the results. Moreover, depending on the test system employed/the manufacturer the results differ strongly. In order to appraise the antibody concentration, determination of the end-titres via various dilution steps is necessary. Overall, the MIF is restricted in terms of requirements of routine laboratory diagnosis.

Enzyme immunoassay

In the routine laboratory automatable ELISAs have become accepted. In order to assess the progress of disease and treatment success, respectively, in a better way, monitoring of antibody courses is necessary. In chronic infections constant antibody concentrations are characteristic. In contrast, antibody concentrations increase significantly in acute primary infections and reinfections. However, real changes in antibody concentrations can only be ascertained by a reliable quantification. The semi-quantitative interpretation of results in terms of cut-off index (e.g., with the species-specific **Chlamydia trachomatis-IgG** and **IgA-pELISA medac** and **Chlamydia pneumoniae-IgG** and **IgA-sELISA medac**) has been a first step towards quantification. The single-point calibration/single point quantification which is used in both species-specific **Chlamydia trachomatis-IgG** and **IgA-ELISA *plus* medac** and **Chlamydia pneumoniae-IgG** and **IgA-ELISA *plus* medac** constitutes an essential progress in regard to quantification.

The medac Single-Point Quantification

Technical advantages

No need for a standard curve

Correction of the measured values via calibrator

Rapid, software-assisted analysis of the results

High reproducibility of the results
Better comparability of the results

“State of the Art” diagnosis

Clinical advantages

Reliable monitoring of antibody concentrations
owing to better standardisation

Reliable antibody courses for assessment of disease states

Reliable antibody courses for assessment of treatment success

Clear statements

Acute diseases (primary infections, reinfections)
Chronic diseases

C. pneumoniae and C. trachomatis ELISA *plus* medac

Quantitative interpretation via single-point quantification (SPQ)

Prerequisite:

Controls	Validity Criteria
Blank	OD* < 0,100
Negative Control	OD < 0,100
Positive Control	Defined AU/ml** nominal range
Calibrator	Lower OD borderline value

* Optical density
** Batch-specific

Calculation of antibody concentrations for IgG and IgA

Correction of the measured values

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

Unit Calculation

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

AU = arbitrary Unit
The curve parameters a and b are batch-dependent.

The single-point quantification represents an user-friendly, economical method for the calculation of quantitative results. The medac single-point quantification is easy to perform, easy programmable and, software-assisted, rapidly analysable.

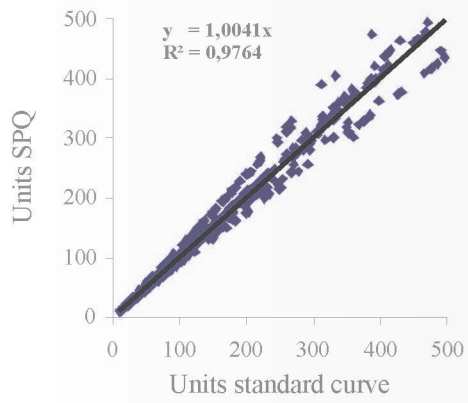
Determination of Antibody Concentrations

Standard curve versus calibrator

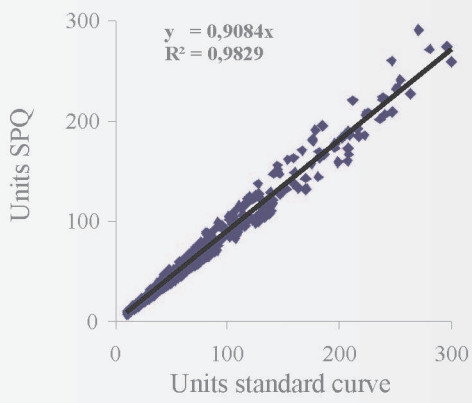
The single-point quantification (SPQ) from medac is a highly precise method which provides results that are equivalent to a conventional standard curve (see graphs below).

C. pneumoniae

IgG

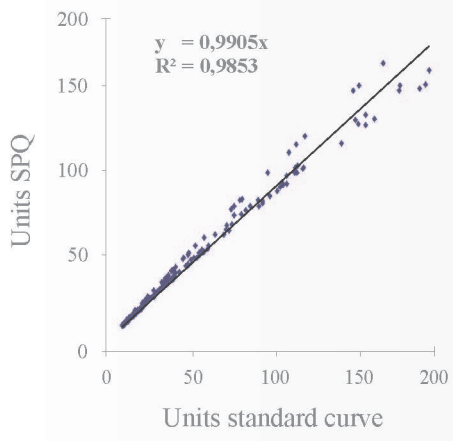


IgA

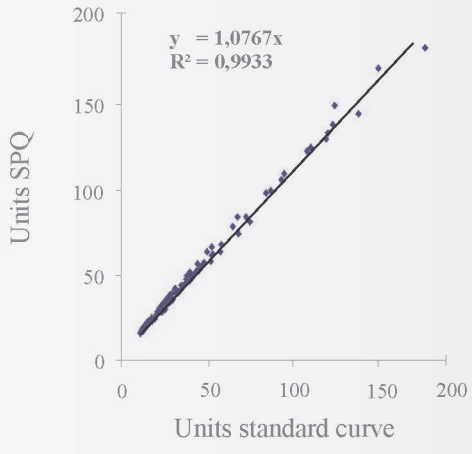


C. trachomatis

IgG



IgA



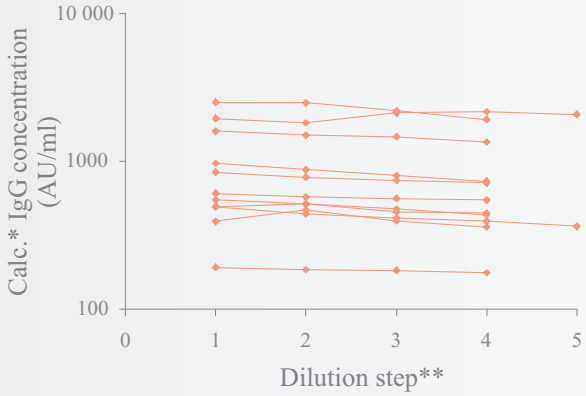
Determination of Antibody Concentrations

Dilution linearity

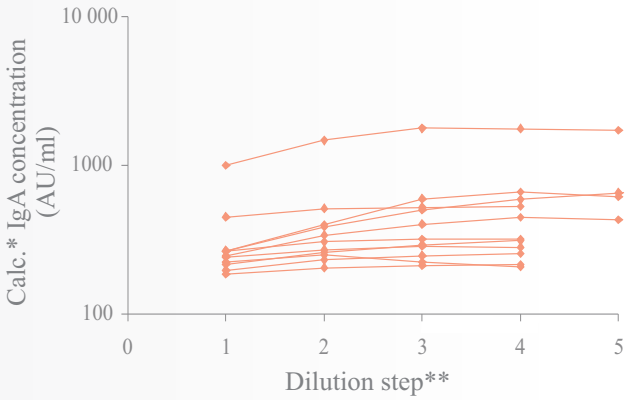
Dilution linearity is an essential prerequisite for monitoring the course of high-titre sera. Both the **Chlamydia pneumoniae IgG** and **IgA ELISA *plus* medac** and the **Chlamydia trachomatis IgG** and **IgA ELISA *plus* medac** fulfil these requirements very well (see graphs below).

C. pneumoniae

IgG

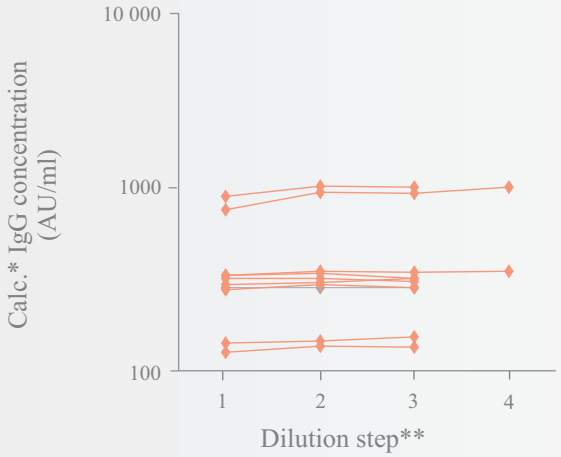


IgA

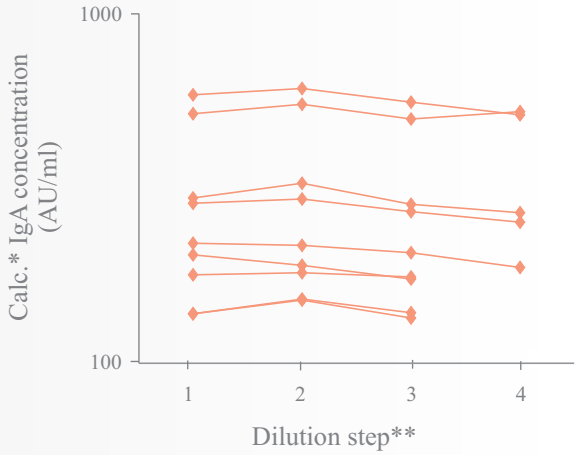


C. trachomatis

IgG



IgA



* Calculated antibody concentration
** in each case 1:2

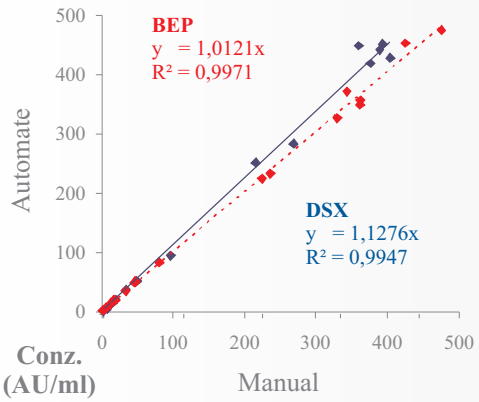
Possibility of Automation

Automated versus manual test runs

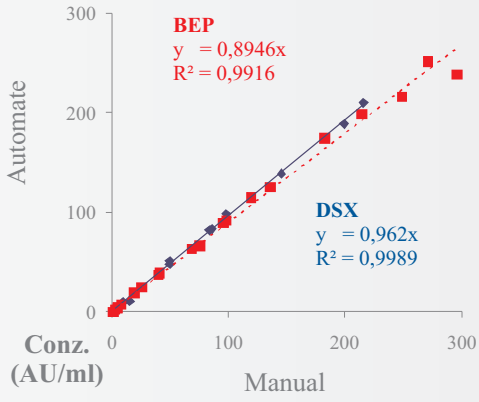
The possibility of automation of the **Chlamydia pneumoniae IgG** and **IgA ELISA *plus* medac** and **Chlamydia trachomatis IgG** and **IgA ELISAs *plus* medac** was assessed with various opened systems/ELISA processors. The concordance between automated and manual test procedures is reflected by the good correlation of the results (see graphs below).

C. pneumoniae

IgG

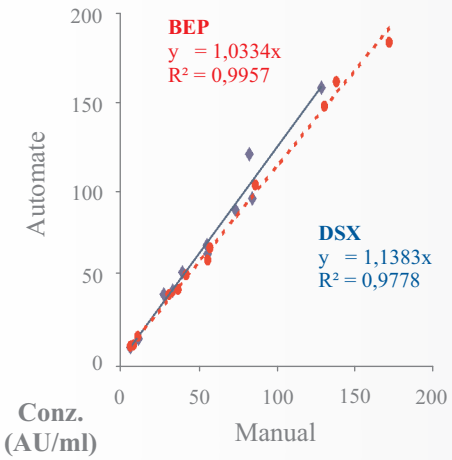


IgA

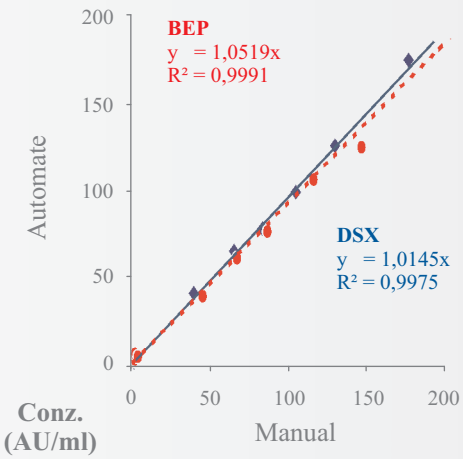


C. trachomatis

IgG



IgA



Precision data

Intra-assay and interassay variations

For assessment of intra-assay and interassay variation five sera of different reactivity were measured (manual test runs).

To establish intra-assay variation, these sera were each tested in a 21-fold assay. To establish interassay variation, these sera were each tested by 11 independent test runs (see tables below).

Intra-assay variation

C. pneumoniae

Sera	IgG		IgA	
	AU/ml	VC	AU/ml	VC
	61	5%	47	5%
	13	4%	31	10%
	55	8%	68	7%
	142	9%	103	8%
	397	10%	186	7%

C. trachomatis

Sera	IgG		IgA	
	AU/ml	VC	AU/ml	VC
	5	7%	4	9%
	41	2%	58	4%
	51	5%	92	3%
	90	5%	121	5%
	110	5%	264	6%

Interassay variation

C. pneumoniae

Sera	IgG		IgA	
	AU/ml	VC	AU/ml	VC
	75	8%	52	4%
	13	9%	29	6%
	59	8%	71	5%
	120	7%	148	5%
	432	7%	208	5%

C. trachomatis

Sera	IgG		IgA	
	AU/ml	VC	AU/ml	VC
	9	5%	3	11%
	32	5%	41	5%
	40	3%	71	5%
	115	7%	96	5%
	151	6%	125	7%

Sensitivity and Specificity

Semi-quantitative versus quantitative analysis

For assessment of sensitivity and specificity of the **Chlamydia pneumoniae IgG** and **IgA ELISA *plus* medac** and the **Chlamydia trachomatis IgG** and **IgA ELISA *plus* medac** the corresponding semi-quantitative, approved and routine-proven **Chlamydia pneumoniae IgG** and **IgA sELISA medac** and **Chlamydia trachomatis IgG** and **IgA pELISA medac** were used as reference (see 4-square tables below). Sensitivity and specificity of the ELISA *plus* generation were assessed using an uniform cut-off of 25 AU/ml for all *plus* ELISAs.

C. pneumoniae

C. pneumoniae sELISA

C. pneumoniae *plus*

IgG	-	+
-	85	0
+	0	206

Specificity	100%
Sensitivity	100%
Correlation	100%

IgA	-	+
-	119	0
+	0	113

Specificity	100%
Sensitivity	100%
Correlation	100%

C. trachomatis

C. trachomatis pELISA

C. trachomatis *plus*

IgG	-	+
-	172	2
+	2	128

Specificity	99%
Sensitivity	98%
Correlation	99%

IgA	-	+
-	243	0
+	3	63

Specificity	99%
Sensitivity	100%
Correlation	99%