

***Chlamydia trachomatis* Infections  
of the Upper Genital Tract**

**and Serology with**

**Chlamydia trachomatis-IgG- and IgA-pELISA  
medac**

**A Mini-Review**

**Including the Diagnostic Value of the Detection  
of Local anti-*C. trachomatis* IgA Antibodies  
in Seminal Fluid from Subfertile Men**

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## **Chlamydia trachomatis Infections of the Upper Genital Tract and Serology with Chlamydia trachomatis-IgG- and IgA-pELISA medac**

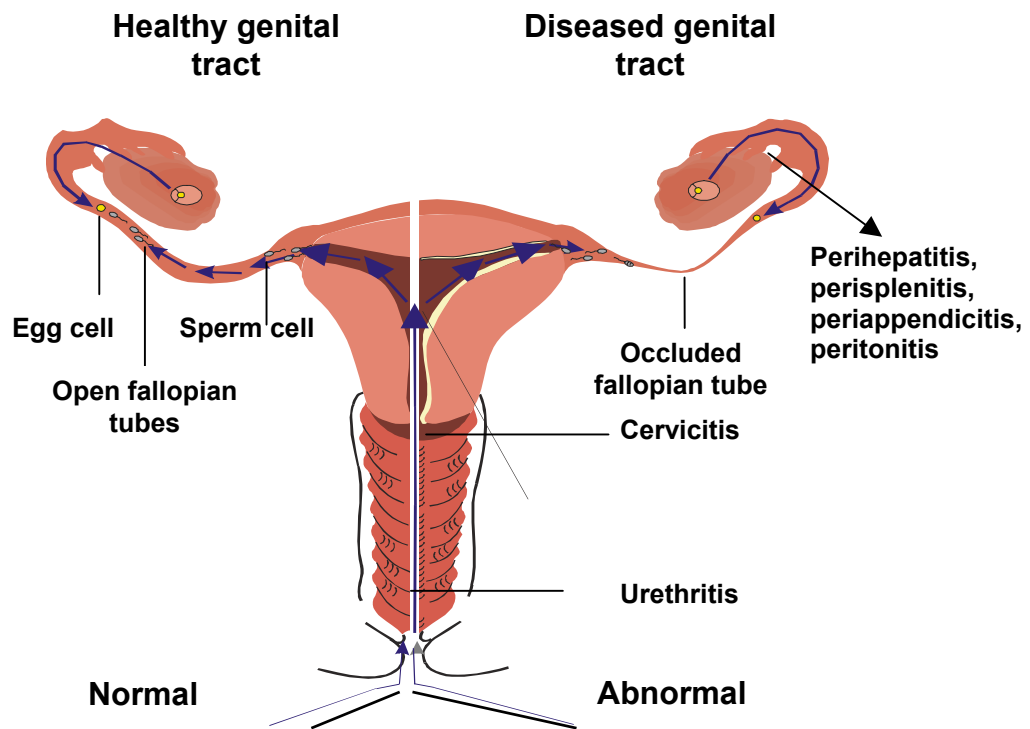
### **Epidemiology and prevalence**

*C. trachomatis* has a worldwide distribution. It is the most common sexually transmitted bacterium. The majority of infected women as well as a large proportion of infected men are asymptomatic. As such, the infection is continuously spread, especially in sexually active and high risk populations.

### **Incidence**

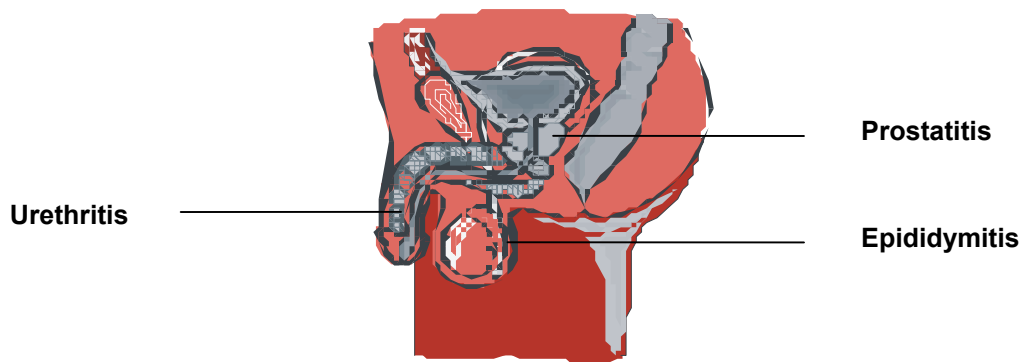
*C. trachomatis* is estimated by the World Health Organization (WHO) to have infected 92 million persons worldwide in 1999. This high burden of disease in both industrialized and developing countries leads to severe consequences related to morbidity and mortality in the populations, as well as adding to the economic burden on healthcare delivery.

### **Chlamydia trachomatis infections in women**



The site of entry in women is the cervix where *C. trachomatis* may cause acute inflammations (cervicitis). Undiagnosed or insufficiently treated cervical *C. trachomatis* infections take a chronic course. The pathogens ascend via uterus to the fallopian tubes and ovaries. This may induce repeated or long-term inflammations leading to pelvic inflammatory disease (PID), salpingitis, and ovarian abscesses. In patients suffering from salpingitis, repeated inflammation in the tubes with subsequent scarring either impairs or blocks the tubal function which results in ectopic pregnancy (EP) and tubal factor infertility (TFI), respectively. *C. trachomatis* is the most frequent cause of acquired infertility. Furthermore, *C. trachomatis* may spread to the abdominal cavity causing perihepatitis, perisplenitis, periappendicitis and peritonitis.

## **Chlamydia trachomatis infections in men**



There has been less research in men on the complications of chlamydial infections than in women. Evidence suggests that after undiagnosed or insufficiently treated urethritis ascended *C. trachomatis* may cause chronic abacterial prostatitis and epididymitis (especially in younger men), as well as inflammation and scarring of the seminiferous tubules and the associated ducts. In the extreme case this may lead to obliterations which may impair or even inhibit the passage of spermatozoa (occlusion azoospermia) even if they are still produced and, thus, may result in chlamydia-induced male infertility.

### **Chlamydia trachomatis transmission**

The common mode of transmitting *C. trachomatis* infections is unprotected sexual intercourse. In addition to the course of infection as described above for women and men, there may be other peculiarities in male infections:

It was observed that *C. trachomatis* was attached to spermatozoa recovered from the peritoneal cavity of patients with salpingitis and that they also enter into human spermatozoa. It was concluded that spermatozoa may serve as vectors for direct, deep transmission of the pathogens to the female reproductive tract with the described subsequent complications.

*C. trachomatis* has been shown to survive cryopreservation in infected human donor semen. Artificial insemination of *C. trachomatis* contaminated cryopreserved human semen may adversely affect the physical and reproductive status of the potential mother, normal *in-vitro* embryo development or even the outcome of *in vitro*-fertilization.

### **Chlamydia trachomatis persistence**

Undiagnosed or insufficiently treated *C. trachomatis* infections may convert to a persistent state. Whilst in this state, the intracellular life cycle is characterized by the development of atypical, metabolically inert and, thus, antibiotic resistant inclusions. This biologic behaviour correlates with the clinical course after acute symptomatic disease, namely persistence or recurrence of symptoms which are not treatable with antibiotics. During the persistent state *C. trachomatis* down-regulates the expression of the major outer membrane protein (MOMP), continues to express lipopolysaccharide (LPS) and over-expresses heat shock protein 60 (HSP60). HSP60 is a highly immunogenic protein that has been implicated in the pathogenesis of chronic inflammatory chlamydial diseases.

## Diagnosics

Culture, direct antigen detection (IFA, ELISA) or nucleic acid amplification techniques (NAAT), and serology complement one another depending on the infection stage that is being investigated. In acute, peripheral, urogenital infections, direct antigen detection or NAAT are the methods of choice. For epidemiological investigations, i.e., previous infection with the pathogen or possible persistent infections, *C. trachomatis* serology should be used (Table 1).

Age Group (years)	Prevalence of <i>Chlamydia trachomatis</i> Asymptomatic Population			
	Women		Men	
	Antigen	IgG	Antigen	IgG
15-24	6.6%	7%	8.7%	3.0%
25-34	2.2%	14%	3.8%	15%
35-39	1.7%	27%	2.0%	13%
40-50	0.5%	20%	0.8%	14%

Table 1: Prevalence of *C. trachomatis* antigen and antibodies in the asymptomatic population

In chronic female upper genital tract infections, in which the pathogens have already ascended, the demonstration of the pathogen in peripheral samples is of limited diagnostic value. Invasive sampling from upper genital tract areas may not provide material for a reliable diagnosis because of the patchy distribution of the pathogen. Here, serology is of value (Table 2).

Prevalence of Antigen and Antibodies in Various Selected Cohorts			
	Antigen	IgG	IgA
<b>Women</b>			
Obviously healthy (age 15-65 years)	3%	17%	5%
Pregnant women	2%	20%	5%
Prostitutes	4%	75%	37%
Women with TFI	< 1%	> 60%	> 20%
<b>Men</b>			
Obviously healthy (age 15-65 years)	4%	12%	7%
<b>Women and Men</b>			
Blood donors	n.d.	14%	6%
Reactive arthritis	1-3%	48%	41%

TFI: Tubal factor infertility (occluded tubes); n.d.: not determined

Table 2: Prevalence of *C. trachomatis* antigen and antibodies in various selected cohorts

As clearly shown in Tables 1 and 2, antigen detection in the asymptomatic population as well as in various selected patient cohorts identifies just the tip of the iceberg.

In peripheral acute male genital tract infections like acute urethritis, NAAT should be used with preference (Table 3).

In male upper genital tract infections, the detection of local anti-*C. trachomatis* IgA antibodies in seminal fluid seems to provide more reliable diagnosis of *C. trachomatis* infections than NAAT (Table 3).

It has been accepted that the identification of *C. trachomatis* in semen by NAAT/cell culture often leads to false negative results due to the presence of inhibitors/toxic substances in this material (Table 3).

Cohorts	n	NAAT Semen	Local IgA	Serum IgG	Serum IgA	Author
Patients with acute urethritis	23	12% (urine)	n.d.	n.d.	n.d.	Bollmann et al. (2001)
Patients without acute urethritis	101	0%	7%	26%	15%	Ludwig et al. (1996)
Male partners of infertile couples	77	4%	n.d.	n.d.	n.d.	Bollmann et al. (2001)
Andrologic patients	834	4%	38% <sup>g</sup>	68% <sup>g</sup>	19% <sup>g</sup>	Bollmann et al. (2001)
Andrologic patients	179	2.8%	13% <sup>s</sup>	n.d.	n.d.	Bollmann et al. (2001)
Andrologic patients	125	1.6%	9% <sup>g,s</sup>	30% <sup>g</sup>	9% <sup>g</sup>	Ochsendorf et al. (1999)
Male partners of infertile couples	197	0%	19% <sup>g</sup>	28% <sup>s</sup>	n.d.	Eggert-Kruse et al. (1996)
Male adnexitis patients	205	1.8%	29% <sup>g</sup>	n.d.	n.d.	Wolff et al. (1994)

n.d.: not determined; g: genus-specific; s: species-specific

Table 3: Value of local anti-*C. trachomatis* IgA and serum IgG antibody determinations compared with NAAT in various male cohorts with upper genital tract infections

The broad range of positive antibody results in seminal fluid and serum samples in Table 3 can be explained by use of various test systems, both genus-specific and species-specific ones and, moreover, by various, non-standardized, non-validated dilutions of seminal fluid.

Significant associations between local anti-*C. trachomatis* IgA and serum IgG had been described by three authors:  $p < 0.05$  by Ludwig,  $p = 0.0002$  by Ochsendorf,  $p < 0.001$  by Eggert- Kruse.

In recent time the significance of local anti-*C. trachomatis* IgA antibodies in male upper genital infections and infertility became clearer.

In regard to male upper genital tract infection, detection of local anti-*C. trachomatis* IgA antibodies in seminal fluid helps to identify occult, silent *C. trachomatis* infections and the corresponding risk of transmission of the pathogens via contaminated spermatozoa to the female partners.

Concerning male infertility, the opinion is still widespread that there is no association between *C. trachomatis* infections in the upper genital tract and male infertility. One reason is the scarce rate of positive PCR results in semen of infertile men ( $\leq 5\%$ ). Another reason may be attributed to the fact that in various studies no statistical significant associations could be found between the presence of *C. trachomatis* infections and sperm pathology.

However, contrariwise significant associations between local anti-*C. trachomatis* IgA antibodies in seminal fluid and various sperm parameters had been demonstrated. Table 4 shows the association between such antibodies and inflammation parameters (elastase, peroxidase) as well as with a marker for prostate activity (citric acid).

Inflammation parameters	Local IgA	p-Value	Author
Granulocyte elastase ↑↑ Citric acid ↓↓	positive	significant	Wolf et al. (1991)
Granulocyte elastase ↑↑	positive	0.009	Ochsendorf et al. (1994)
Peroxidase-positive leukocytes ↑↑	positive	< 0.01	Weidner et al. (1996)
Granulocyte elastase ↑↑	positive	< 0.04	Ochsendorf et al. (1999)
Peroxidase-positive leukocytes ↑↑	positive	0.002	Pannekoek et al. (2003)

Table 4: Association between local anti-*C. trachomatis* antibodies in seminal fluid and inflammation parameters

In Table 5 significant associations between local anti-*C. trachomatis* IgA antibodies in seminal fluid and sperm pathology are displayed.

Semen pathology	Local IgA	p-Value	Author
Anti-sperm antibodies	positive	<0.0001	Witkin et al. (1995)
Reduced sperm count	positive	0.003	Witkin et al. (1995)
Reduced acrosome reaction	positive	significant	Jungwirth et al. (2003)
Pathospermia	positive	significant	Rezacova et al. (2004)

Table 5: Association between local anti-*C. trachomatis* antibodies in seminal fluid and sperm pathology

The reason why in total such discrepant results exist, cannot be explained. However, as further displayed on pages 8 and 9, it is nowadays justified to integrate the detection of local anti-*C. trachomatis* IgA antibodies in seminal fluid into the basic screening of male infertility.

## Chlamydia trachomatis-IgG- and IgA-pELISA medac

### Technical data

Antigen:

The antigen employed is a synthetic peptide from the major outer membrane protein (MOMP) of *C. trachomatis*.

Reproducibility:

A high reproducibility for both intra- and interassay variation was found during medac's validation and independently confirmed by an external investigator (Table 6).

	Intra-Assay Variation				Interassay Variation			
	medac		external <sup>1</sup>		medac		external <sup>1</sup>	
Antibody iso-type	n	Mean CV %	n	Mean CV %	n	Mean CV %	n	Mean CV %
IgG	21	3.2	18	2.7	21	8.5	18	13.6
IgA	21	5.3	18	3.3	21	8.0	18	5.5

<sup>1</sup>: Verkooyen et al. (2000)

Table 6: Intra- and interassay variation of the Chlamydia trachomatis-IgG- and IgA-pELISA medac

Specificity:

Investigations of various cohorts with IgG and IgA pELISA revealed a high specificity as shown in Table 7.

Cohorts	n	Specificity	
		IgG	IgA
Children (< 10 years) (medac)	100	99.0%	98.0%
MIF antibody positive for <i>C. pneumoniae</i> (medac)	47	100%	100%
Significant titer increases of <i>C. pneumoniae</i> antibodies (Verkooyen et al. 2003)	22	100%	100%

Table 7: Specificity of the Chlamydia trachomatis-IgG- and IgA-pELISA medac

Data on prevalence of IgG and IgA antibodies as detected with IgG and IgA pELISA in various cohorts are summarized in Table 8 and additionally in Table 9 for males including the presence of local anti-*C. trachomatis* IgA antibodies in seminal fluid.

As can be seen from Table 8, pELISA discriminates well between low risk and high risk groups. The results obtained by different study groups correlate with previously published data (Table 2).

Cohorts	n	pELISA Antibody Prevalence	
		IgG	IgA
Blood donors (medac)	299	15%	6%
Blood donors (Verkooyen et al. 2002)	443	12%	5%
Blood donors (Petersen et al. 2002)	39	8%	3%
Blood donors (Ziegert et al. 2000)	165	11%	3%
Blood donors (Petersen et al. 2000)	316	14%	4%
Pregnant women (Petersen et al. 2000)	146	12%	3%
Infertile women with patent tubes (Verkooyen et al. 2002)	49	12%	4%
STD outpatients, culture neg. (medac)	112	25%	6%
STD outpatients, PCR neg. (Verkooyen et al. 2002)	100	38%	17%
STD outpatients, LCR neg. (Spaargaren et al. 2002)	224	27%	n.d.
PID patients, LCR neg. (Petersen et al. 2002)	35	23%	14%
STD outpatients, culture pos. (medac)	114	65%	25%
STD outpatients, PCR pos. (Verkooyen et al. 2002)	324	75%	45%
STD outpatients, LCR pos. (Spaargaren et al. 2002)	106	77%	n.d.
PID patients, LCR pos. (Petersen et al. 2002)	54	78%	33%
IVF patients, unselected (Petersen et al. 2000)	127	33%	11%
Women with primary infertility (Ziegert et al. 2000)	288	32%	32%
Women with secondary infertility (Ziegert et al. 2000)	192	48%	13%
Women with occluded tubes (Verkooyen et al. 2002)	85	55%	24%
Women with occluded tubes (Petersen et al. 2002)	27	74%	26%
Women with occluded tubes (Petersen et al. 2000)	85	60%	15%
Women with occluded tubes (Dale et al. 2000)	12	75%	n.d.

n.d.: not determined

Table 8: Prevalence of anti-*C. trachomatis* antibodies in various patient cohorts as detected with pELISA

The results on the prevalence of local anti-IgA antibodies in seminal fluid obtained with pELISA versus NAAT (Table 9) underline the findings on the low diagnostic value of NAAT in this material which are presented in Table 3. In the future, screening of andrologic patients as well as of semen donors for local IgA antibodies instead with NAAT should be considered as alternative in order to avoid transmission to the females and possible adverse outcome of IVF.

When compared to other serological test systems, pELISA has the unique advantage of a thoroughly validated, standardized dilution of seminal fluid and a defined cut-off.

Cohorts	n	pELISA Antibody Prevalence				
		Semen	Seminal fluid		Serum	
		NAAT	IgA	IgG	IgG	IgA
Blood donors (Schuppe et al. 2002)	136	n.d.	n.d.	n.d.	8%	7%
Subfertile men (Schuppe et al. 2002)	151	n.d.	13%	n.d.	18%	n.d.
Andrologic patients (Bollmann et al. 2001)	105	2.9%	18.7%	n.d.	n.d.	n.d.
Subfertile men (Schuppe et al.2000)	171	4.7%	26.9	n.d.	17%	12.3%
Semen donors (Bollmann et al. 2001)	47	0%	6.4%	n.d.	n.d.	n.d.
Semen donors (Schuppe et al. 2000)	116	0%	9.5%	n.d.	n.d.	n.d.

n.d.: not determined

Table 9: Prevalence of anti-*C. trachomatis* antibodies in serum and seminal fluid of various male patient cohorts as detected with pELISA

### Clinical findings

In addition to these technical data the association between local anti-*C. trachomatis* IgA antibodies in seminal fluid and pathospermia also has been confirmed in a big private andrological laboratory which has been using pELISA since years (Labor Dr. Furrer AG, Zurich, Switzerland) and which had performed a first analysis of recent data. The determination of local anti-*C. trachomatis* IgA antibodies is performed in connection with routine diagnosis in patients with infertility as well as in patients with various urological problems, such as prostatitis, epididymitis, obstructions of the seminal passages etc.

In infertile patients, local anti-*C. trachomatis* IgA antibodies are determined in parallel to the spermograms according to the WHO criteria and to *C. trachomatis* PCR. In patients with various urological problems, the presence of this antibody isotype is examined in connection with semen cultures for general bacteria, *Mycoplasma hominis*, *Ureaplasma urealyticum* and with *C. trachomatis* PCR.. An analysis on the routine results obtained between January 2004 and September 2004 was performed (Table 8).

In general, high associations were found between partly extremely high local anti-*C. trachomatis* IgA antibody levels in seminal fluid and pathological sperm morpho

logy in infertile patients. In various patients the frequency of pathological spermatozoa forms exceeded 90% (Table 8).

In individual cases additional associations with other semen parameters were found as displayed in Table 10.

<b>Patient characteristics</b>	<b>Infertility</b>
<b>Number of spermograms</b>	<b>541</b>
<b>IgA antibodies in seminal fluid (%)</b>	<b>72/541 (13.3%)</b>
<b>PCR positivity in semen</b>	<b>≤5%</b>
<b>General association with pathospermia</b>	<b>aberrant morphology, partly &gt;90%</b>
<b>Individual association with pathospermia</b>	<b>anti-sperm antibodies sperm density ↓↓ sperm count ↓↓ sperm motility ↓↓ pathological concentrations of biochemical markers</b>

Table 10: Analysis of results obtained at the laboratory Dr. Furrer between January 2004 and September 2004

Analyzing the results obtained in the patients with various urological problems over the same period, January 2004 until September 2004, a prevalence of local anti-*C. trachomatis* IgA antibodies in seminal fluid was obtained in 37/184 patients (20.1%). Also in these patients the positive *C. trachomatis* PCR detection rate was ≤5%.

Like various other laboratories which use the IgA pELISA for detection of local anti-*C. trachomatis* IgA antibodies in seminal fluid, this laboratory advises treatment of all patients who are positive for local anti-*C. trachomatis* antibodies as well as obligatory treatment of the partners for at least 14 days with doxycycline. Alternatively, treatment with macrolides (clarithromycin or azithromycin) is suggested.

According to the longtime experiences of this laboratory, the detection of local anti-*C. trachomatis* IgA antibodies has been proven itself to be the most reliable indicator of ascended *C. trachomatis* infections. Furthermore, treatment of the IgA-positive patients results in improvement of the clinical picture as well as in increased fertility rates.

### Summary

- *C. trachomatis* is the most common sexually transmitted bacterium.
- Infections with *C. trachomatis* take a predominantly asymptomatic course.
- *C. trachomatis* contaminated spermatozoa may be the ideal vector for rapid, deep transmission of the pathogen to female partners.
- Undiagnosed and, thus, untreated *C. trachomatis* infections lead to persistence of the pathogens in the upper genital tract.
- The most frequent sequelae of persistent pathogens are those of the reproductive system (ectopic pregnancy, infertility).
- NAAT are the methods of choice for diagnosis of acute peripheral *C. trachomatis* infections (urethritis, cervicitis).

- In chronic upper genital tract infections the detection of *C. trachomatis* in peripheral samples may lead to false negative results as the pathogens already may have ascended.
- In chronic upper female genital infections *C. trachomatis*-specific serology has been considered method of choice.
- *C. trachomatis*-conditioned fertility problems in females have been generally accepted, and the serological detection of anti-*C. trachomatis* antibodies has been widely used in connection with the *in vitro* fertilization (IVF) basis screening.
- In chronic upper genital male infections the determination of local anti-*C. trachomatis* IgA antibodies in seminal fluid provides more information on the presence of *C. trachomatis* than NAAT.
- Detection of local anti-*C. trachomatis* IgA antibodies in seminal fluid helps to identify occult, silent *C. trachomatis* infections and the corresponding risk of transmission of the pathogens via contaminated spermatozoa to the female partners.
- In regard to male infertility and other diseases of the upper genital tract significant associations between local anti-*C. trachomatis* IgA antibodies in seminal fluid and inflammation parameters as well as pathospermia have been found.
- Appropriate treatment of patients with local anti-*C. trachomatis* IgA antibodies in seminal fluid ameliorated or even cured the state of disease of the patients and increased the fertility rates, respectively.
- In the future, testing of male patients with diseases of the upper genital tract as well as with infertility for the presence of local anti-*C. trachomatis* IgA antibodies in seminal fluid should be added on a broad basis to the routine semen analyses.

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