

Chlamydia pneumoniae

Specific Diagnosis Chlamydia pneumoniae IgG-, IgA-, IgM-sELISA

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Mode of life

Chlamydia pneumoniae belongs to those gram-negative bacteria which are obligate intracellular energy parasites and which colonise in a first step the epithelial cells of the respiratory tract. They are distributed by monocytes in the circulation and can be detected in the plaques of atherosclerotic arteries, in the synovia and even in nerve tissue (1, 2, 3, 4, 5).

Reproduction, infection & treatment

C pneumoniae has a biphasic multiplication cycle characterised by the transition of the infectious but non-replicating elementary body into the non-infectious, replicating reticulate body.

The pathogen is transmitted from person to person by droplet infection.

The incubation period for **C. pneumoniae** infections is approximately 3-4 weeks. In most cases the infection runs a subclinical course or produces symptoms resembling those of influenza, and many infections consequently remain unrecognised. This results in chronic infections with serious sequelae. The inflammatory reactions are repeatedly triggered by frequent reinfections.

Only in the reticulate stage is the pathogen susceptible to antibiotic therapy. Tetracyclines, macrolides and quinolones can all provide adequate therapy. The necessary duration of treatment will depend on the course of the illness. Poor compliance or failure to complete the course will lead to persistence of the pathogen (2).

Dissemination, prevalence & incidence

C pneumoniae is of worldwide distribution. The seroprevalence of **C. pneumoniae** IgG antibodies is age-dependent: in preschool children it is well below 10%, then rising abruptly to about 20-30% between 5 and 14 years of age, and reaching about 50% by the age of 20. From then on the prevalence increases slowly, exceeding 75% beyond the age of 70.

After puberty, a sex-dependent difference in prevalence has been observed, considerably higher in men than in women. This difference has not yet been satisfactorily explained.

The incidence of acute **C. pneumoniae** infections reaches its highest level at 9% per year in children between the ages of 5 and 9 years. After puberty it drops to 1-2%. Epidemics of **C. pneumoniae** infection arise every 4-10 years (6, 7).

Diagnosis

The diagnosis of **C. pneumoniae** infection can be based on direct microscopy and serology. Tests for the pathogen or antigen often give false negative results. Attempts to isolate the pathogen by culture are subject to numerous interfering factors. A standardised, commercial **C. pneumoniae** PCR is not available.

Serology is the method of choice for the diagnosis of **C. pneumoniae** infections. The microimmunofluorescence test (MIF), despite having to be evaluated subjectively, is accepted as the gold standard (2), although it has not been standardised. To date, the species-specific ELISA tests play the major role.

Clinical Manifestations

Atypical pneumonia, bronchitis and sinusitis are the most frequent respiratory diseases caused by *C. pneumoniae*. The infection often begins quite uncharacteristically with pharyngitis, a simple cold in the nose or a cough. The course of the infection is protracted.

Respiratory tract diseases

C. pneumoniae is the cause of 10% 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, though its frequency has not yet been determined (2, 8).

Primary diseases

Most *C. pneumoniae* infections run a chronic course. The complications or sequelae are often more serious than the primary infection. The late sequelae of a *C. pneumoniae* infection may be both pulmonary and extrapulmonary. Long-term persistence of pathogens in the lung may pave the way for chronic obstructive airways diseases: *C. pneumoniae* has been considered as a possible cause of asthma, sarcoidosis and even lung cancer (2, 9, 10, 11).

Sequelae

The extrapulmonary diseases in whose pathogenesis *C. pneumoniae* is allegedly involved can be roughly divided into the following categories: reactive diseases, atherosclerosis and diseases of the central nervous system.

In patients with reactive arthritis, vasculitis, myocarditis and endocarditis, the pathogens have been identified in the relevant tissues and antibodies against *C. pneumoniae* have been identified serologically (2).

Reactive diseases

After Saikku et al. (1988) published their paper in The Lancet suggesting that *C. pneumoniae* might be involved in the etiology of coronary heart disease or acute myocardial infarction, the pathogen was also identified in arteriosclerotic plaques by several other groups. The pathogen was found to be capable of cultivation and hence treatable. An association between *C. pneumoniae* infections and coronary heart disease has been established in even more numerous cases by serological testing.

Coronary heart diseases

The prospective therapeutic trials at present in progress may conceivably confirm the hypothesis that plaque formation in the arteries is of infective origin (3, 12, 13, 14, 15, 16).

As regards the etiology of multiple sclerosis, the principal demyelinating disease of the CNS, recent work shows that *C. pneumoniae* cannot be excluded as a possible cause. The pathogens have been identified in cerebrospinal fluid, as have been IgG antibodies against *C. pneumoniae* (17, 18).

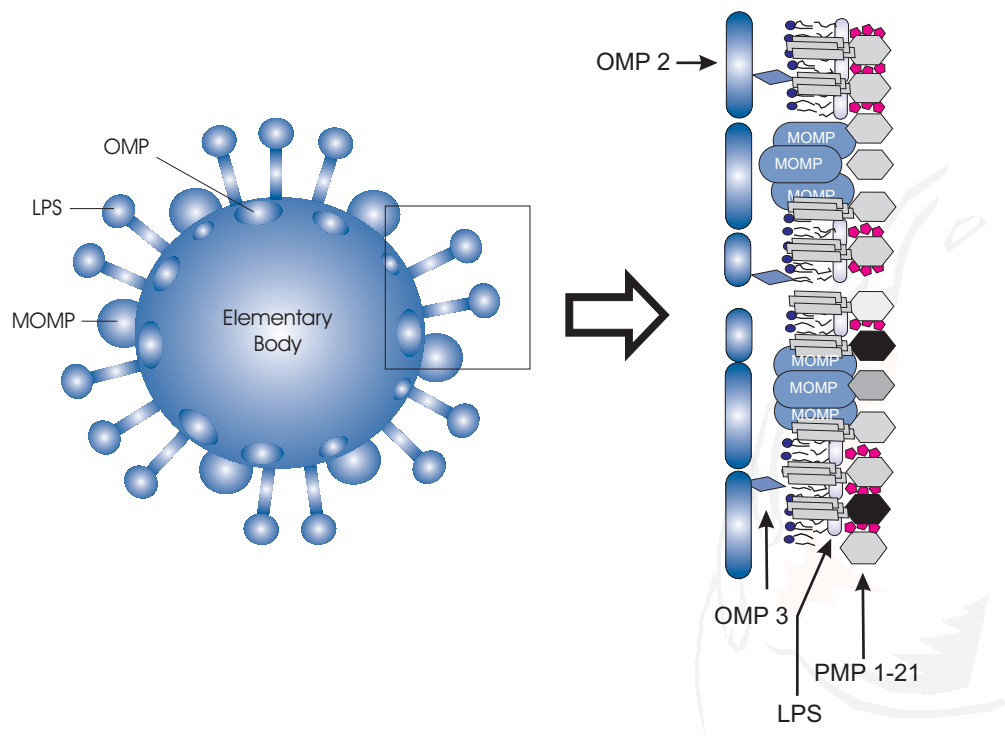
Diseases of the central nervous system

C. pneumoniae is likewise under suspicion as a risk factor responsible for the neuropathological changes in late type Alzheimer's disease. Specific DNA has already been detected in relevant brain areas. IgG antibodies against the pathogen have been detected in cerebrospinal fluid. The control group showed significant differences in both parameters (5).

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Antigenic structures

In all species of chlamydia known to be human-pathogenic, the cell wall of the microorganism (Figure*) consists of the genus-specific lipopolysaccharide (LPS) and numerous Outer Membrane Proteins (OMPs). Among these membrane proteins the major outer membrane protein (MOMP = OMP1) has a predominant share amounting to roughly 60%.



Although the basic structure of the cell wall is the same in all chlamydia, significant species-specific deviations have been recognised among them. For some time the crucial species-specific epitopes of chlamydia have been thought to reside on the variable domains of the MOMP. This has now been confirmed for *C. trachomatis*. The species-specific sequences for *C. trachomatis* have been localised with confidence on the variable domain IV of MOMP. These epitopes now constitute the basis for the specific peptide ELISA tests.

The cell wall structure of *C. pneumoniae* seems to be much more complicated in its details. The major membrane protein does not carry the species-specific epitopes. Furthermore, it is only feebly immunogenic. The species-specific *C. pneumoniae* epitopes are evidently located on the closely folded polymorphic membrane proteins (PMP1-21) (2, 19).

The antigen for the *C. pneumoniae* sELISA medac tests was selected in the light of these new discoveries. The *C. pneumoniae* sELISA test is based on a highly purified native antigen from which LPS has been eliminated.

IgG-, IgA-, IgM-sELISA medac

To determine specificity, measurements were performed on sera from patients in whom there was no clinical suspicion of any respiratory infection. Antibodies against *C. pneumoniae* were **not** detectable by the **MIF** test in **any** of the sera.

Specificity

Specificity			
IgG	IgA ¹	IgM ²	
95% (n = 42)	93 % (n = 86)	90 % (n = 31)	medac
86% (n = 42)	58% (n = 84)	81% (n = 31)	Savyon

To determine sensitivity, assays were performed on sera from patients with clinical suspicion of a respiratory infection. The **MIF** test revealed antibodies against *C. pneumoniae* in **all** these sera.

Sensitivity

Sensitivity			
IgG	IgA ¹	IgM ²	
99% (n = 116)	95 % (n = 74)	97 % (n = 38)	medac
92% (n = 116)	99% (n = 77)	89% (n = 38)	Savyon

¹ Difference in the number of samples due to equivocal sera, which had not been taken into consideration

² Measurements at external laboratories. Additionally, sera from further cohorts were assessed at medac's (see package insert). The specificity for IgM was between 98% and 99%.

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Cross-reactivity



To elucidate any possible cross-reactivity with *C. trachomatis*, 11, 14 and 12 sera respectively were investigated. These sera had already been characterised in the MIF test (MRL) as exclusively *C. trachomatis*-positive.

		C. pneumoniae IgG		
		Savyon		
		-	+	
sELISA medac	-	7	3	
	+	0	1	
				11

		C. pneumoniae IgA		
		Savyon		
		-	+	
sELISA medac	-	3	10	
	+	0	1	
				14

		C. pneumoniae IgM		
		C. trachomatis-IgM-MIF		
		-	+	
sELISA medac	-	0	12	
	+	0	0	
				12

Single IgA



To establish the incidence of solitary IgA, all sera which had been assayed during the validation phase of the *C. pneumoniae*-sELISAs were analysed retrospectively. Solitary IgA results are exceptional findings, which occur very rarely and are independent of the chlamydia species and the test system.

Prevalence			
	n	IgA	single IgA
<i>C. pneumoniae</i> -sELISA medac	1157	48%	1%
SeroCP Savyon	1158	61%	6%

IgG-, IgA-, IgM-sELISA medac

Precision

To establish intra-assay variance, five different sera were each tested in a 21-fold assay.

Intra-assay variance						
Serum	IgG		IgA		IgM	
	OD	CV (%)	OD	CV (%)	OD	CV (%)
1	0.032	(27)	0.026	(42)	0.036	(9)
2	0.772	(5)	0.512	(4)	0.503	(5)
3	1.990	(3)	1.251	(3)	1.216	(4)
4	1.951	(5)	1.419	(4)	0.844	(7)
5	0.731	(4)	0.169	(7)	0.719	(6)

To establish interassay variance, five different sera were each tested by 11 independent test procedures.

Interassay variance						
Serum	IgG		IgA		IgM	
	OD	CV (%)	OD	CV (%)	OD	CV (%)
1	0.035	(11)	0.027	(28)	0.035	(10)
2	0.824	(9)	0.533	(8)	0.521	(5)
3	1.027	(8)	0.663	(7)	1.225	(5)
4	1.655	(8)	0.975	(9)	0.051	(9)
5	2.133	(7)	1.243	(9)	0.848	(7)

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Literature

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