Mycoplasma pneumoniae

Specific and sensitive diagnosis
Mycoplasma pneumoniae
IgG-, IgA-, IgM-ELISA

Mycoplasma pneumoniae

Definition/systematics

▶ *Mycoplasma pneumoniae* belongs to the family Mycoplasmataceae. They constitute together with four other families the self-contained class mollicutes (soft-skinned).

Mycplasmae don't possess a cell wall. They are enclosed by a membrane which is very flexible thus allowing extreme changes of their shape (pleomorphy). Some species, e.g., *Mycoplasma pneumoniae*, possess a structure which resembles to a cytosceleton and which stabilizes the cell shape. With a mean size of 300-800 nm, mycoplasmae represent the smallest bacteria which are capable of autonomous replication. Like viruses they are able to penetrate membrane filters.

Mycoplasmae possess a very small genome (580-2200 kbp) which strongly constricts their synthesising capability.

To date, more than 100 species of the family mycoplasmataceae are known. The majority of them have been found exclusively in animals. In humans, in the meantime, 14 species have been isolated which colonise the mucous epithelia of the respiratory and urogenital tract. Only few of these species are pathogenic.

Mycoplasma pneumoniae belongs to the human-pathogenic species. The agent occurs in two forms which differ in regard to the P1 adhesin.

M. pneumoniae is an obligate extracellular parasite of the mucous epithelia of the respiratory tract which is characterised by high host specificity.

Infection

▶ The infectivity of *M. pneumoniae* is minor. Transmission from human to human takes place by droplet infection.

Firstly, *M. pneumoniae* colonises the mucous epithelia of the respiratory tract. The following virulence factors are decisive: adherence to the epithelia cells, mobility, adaptation to the host, and induction of pathological immune responses. Mycoplasmae adhere to the cilia of the ciliated epithelium. Already few hours later ciliostasis occurs with subsequent destruction of the epithelium. Adherence and colonisation are a pre-condition for further spread of the pathogen in the respiratory tract.

Besides the common respiratory tract infections, *Mycoplasma pneumoniae* may also be responsible for systemic spread. The pathological mechanism which may be responsible for overcoming the epithelial barrier and for entrance of the pathogen into the blood circulation, to date, is not yet clear. Probably the adhesive characteristics of the agent change.

Clinical manifestation

▶ *M. pneumoniae* causes diseases in both the upper and the lower respiratory tract. Besides tracheo-bronchitis and atypical pneumonia, pharyngitis, laryngitis, otitis media, and myringitis may be induced. Meningitis, meningo-encephalitis, polyneuritis (Landry-Guillain-Barré syndrome), myocarditis, pericarditis, myalgia, arthralgia, arthritis, hemolytic anemia, thrombocytopenic purpura as well as affections of the skin (e.g., Stevens-Johnson syndrome) are known extrapulmonary complications and sequelae, respectively.

Epidemiology

▶ *M. pneumoniae pneumoniae* is of worldwide distribution. The pathogen may cause both endemics and epidemics during any season. Epidemics caused by *M. pneumoniae* have been observed every three to seven years.

Infections

Course of disease/therapy

Incubation time after *M. pneumoniae* contamination is between 10 and 21 days. *M. pneumoniae* infections may run both an asymptomatic and symptomatic course. However, the symptoms including fever, dry cough, and headache are very unspecific.

In contrast to infections in adolescents and adults, infections in small children below 5 years of age show a milder course. In small children the infection often is self-limiting.

In general, primary infections seem to take a more favourable course than reinfections. *M. pneumoniae* reinfections normally provoke an inflammatory response which is much stronger than that in primary infections because of auto-immune processes.

After an expired infection persistent pathogens are frequently detectable for weeks or months. The so-called switching, i.e., the spontaneous switching on and switching off of membrane protein synthesis, enables short-dated changes of the membrane's antigenic characteristics. Thus, the pathogen may escape from the present immune response.

The classic therapy includes tetracyclines. Macrolides and chinolones are also used. Because of the lack of a cell wall, cell wall-specific antibiotics are ineffective.

General incidence/prevalence

M. pneumoniae infections belong to the most frequent causes of community acquired tracheo-bronchitis and pneumonia in children and adolescents. The highest incidence is between the 5th and 15th year of age. Up to 20% of all community acquired respiratory diseases may be ascribed to *M. pneumoniae*. In 5-10% of these diseases atypical pneumonia may develop.

The antibody prevalence is age-dependent as determined in a healthy population. In small children the IgG seroprevalence is about 15%, in adults it may reach 40-50%. IgA antibodies are not detectable in small children. In a healthy population of adults up to 30% IgA antibodies have been described.

Diagnosis

As the clinical symptomatology of a *M. pneumoniae* infection does not show pathogen-specific characteristics, the diagnostic differentiation from other pathogens such as viruses and gram-positive bacteria is decisive for appropriate therapy.

The diagnosis can be based on direct detection and serology. Detection of the pathogen has been regarded as efficient diagnosis at acute, early stages of disease.
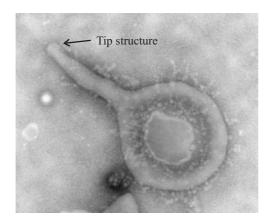
Isolation of the pathogen by culture has been considered the reference method. However, it is too insensitive and time-consuming (6-14 days). A good quality pathogen DNA detection system (PCR) is not yet commercially available.
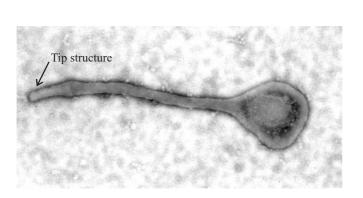
To date, serology has been considered the method of choice for diagnosis of *M. pneumoniae* infections. The complement fixation test (CFT) represents the classic antibody detection. The CFT cannot discriminate between antibody isotypes. Agglutination tests cannot discriminate between antibody classes either. Both test systems detect mainly the IgM antibody response. In reinfections both CFT and agglutination tests provide predominantly negative results. Nevertheless these test systems currently dominate the ELISA technology. By using ELISA, IgG, IgA, and IgM, antibodies can be differentiated. The crucial factor for a specific and sensitive ELISA is the antigen.
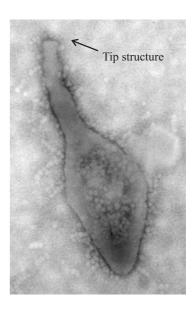
Mycoplasma pneumoniae

Antigenic structure

▶ The adherence of *M. pneumoniae* to the host cell has been regarded as the decisive virulence factor. The docking of the pathogen is effected via the terminal or tip structure which is characteristic for the agent (see figures).

Figures: Electron microscopic pictures of *M. pneumoniae*. By kind permission of Prof. G. Christiansen, Dr. S. Birkelund, University of Aarhus, Denmark, 2004.

The important highly immunogenic proteins in principal are distributed all over the surface of the pathogen. On the terminal structure they may also occur as larger clusters. Out of the more than 10 known immunogenic proteins, the P1 (170 kDa) protein is of predominant importance.

Taking these facts into consideration, the antigens for the **Mycoplasma pneumoniae ELISAs medac** have been selected.
The antigens of the **Mycoplasma pneumoniae-IgG-** and **IgA-ELISA medac** consist of a mixture of various recombinant proteins.
The **Mycoplasma pneumoniae-IgM-ELISA medac** is based on native, purified antigen.

IgG-, IgA-, IgM-ELISA medac

Quantification

The possibility of quantification with **Mycoplasma pneumoniae-IgG-** and **IgA-ELISA medac** provides a good prerequisite for monitoring proven infections whilst in regard to IgM a yes/no result is considered sufficient.

The single-point quantification represents a user-friendly, economic method for ascertainment of quantitative results. The medac single-point quantification is easy to perfom, easily programmable, and the software-based results are rapidly interpretable.

Formula for calculation of concentration

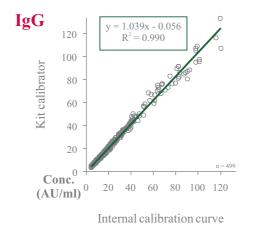$$\text{Concentration [AU/ml]} = b / \left(\frac{a}{OD_{corrected}} - 1 \right)$$

AU = Arbitrary Unit
The curve parameters a and b have to be taken from the batch-specific data sheet.

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

Determination of concentration via standard curve and calibrator (single-point quantification)

The medac single-point quantification is a highly precise method which provides results that are equivalent to a conventional standard curve (see graphs). The measuring range spans from 9 to 130 AU/ml.

IgG — $y = 1.039x - 0.056$, $R^2 = 0.990$, Kit calibrator vs Conc. (AU/ml), n = 499, Internal calibration curve

IgA — $y = 1.034x - 0.963$, $R^2 = 0.993$, Kit calibrator vs Conc. (AU/ml), n = 255, Internal calibration curve

The cut-off is located at 10 AU/ml. The grey zone spans from 9 to 11 AU/ml. Samples with values below the grey zone (< 9 AU/ml) are assessed negative and values beyond the grey zone (> 11 AU/ml) are assessed positive.

Mycoplasma pneumoniae

Sensitivity/specificity

For determination of sensitivity and specificity a reference system is needed. However, in regard to mycoplasma serology no "Gold Standard" exists. This results in the difficulty to determine these parameters. To obtain data in spite of lack of reference tests, a reference system was defined (Reference I) consisting of a particle agglutination test (PAT) and a commercialised ELISA (C-EIA). For determination of sensitivity* and specificity of the medac ELISAs, sera were selected that had provided concordant positive and negative results, respectively, with both PAT and C-EIA. These sera were measured with **Mycoplasma pneumoniae-IgG, IgA-,** and **IgM-ELISA medac** (MP ELISA). All sera with "false negative" results in the medac tests were retested with another commercialised ELISA (Reference II). Considering these results, sensitivity was recalculated (corrected sensitivity).

IgG	Reference I	
MP ELISA	-	+
-	32	6
+	7	38
		83

Specificity	82%
Sensitivity	86%
NPV	84%
PPV	84%
Correlation	84%

Of the 6 sera with "false negative" results in the medac IgG ELISA, 5 were negative and borderline, respectively, when measured with reference II. Considering these measurements, a corrected sensitivity of 98% was calculated.

IgA	Reference I	
MP ELISA	-	+
-	39	13
+	1	20
		73

Specificity	98%
Sensitivity	61%
NPV	75%
PPV	95%
Correlation	81%

Of the 13 sera with "false negative" results in the medac IgA ELISA, 9 were negative and borderline, respectively, when measured with reference II. Considering these measurements, a corrected sensitivity of 88% was calculated.

IgM	Reference I	
MP ELISA	-	+
-	40	8
+	0	31
		79

Specificity	100%
Sensitivity	80%
NPV	83%
PPV	100%
Correlation	90%

Of the 8 sera with "false negative" results in the medac IgM ELISA, all were clearly negative when measured with reference II. Considering these measurements, a corrected sensitivity of 100% was calculated.

* Borderline results were not included into the calculation.

IgG-, IgA-, IgM-ELISA medac
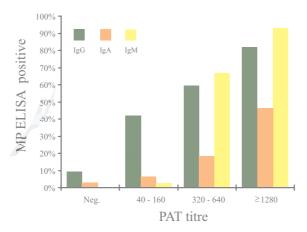
Specific prevalence

To date, the frequency of *M. pneumoniae* infections as well as their serological reflection has been discussed with controversy. To get a current insight, results obtained in sera from three cohorts were compared in MP ELISA and C-EIA: blood donors (general prevalence), hospitalised children, and adults with acute respiratory infections.

Analysing the antibody constellation, sick and healthy cohorts may be well differentiated from each other. The high number of borderline IgG and IgA results obtained with the commercialised C-EIA was striking (see table).

		IgG		IgA		IgM	
		medac	C-EIA	medac	C-EIA	medac	C-EIA
Blood donors (n = 300)	+	39%	30%	6%	7%	2%	1%
	+/-	11%	22%	3%	17%	2%	1%
	-	50%	48%	91%	76%	96%	98%
Hospitalised children (n = 166)	+	67%	63%	9%	13%	28%	35%
	+/-	4%	10%	3%	16%	5%	5%
	-	29%	27%	88%	71%	67%	60%
Hospitalised adults (n = 189)	+	44%	29%	10%	13%	21%	25%
	+/-	5%	14%	3%	3%	5%	1%
	-	51%	57%	87%	84%	74%	74%

The particle agglutination test (PAT) is a screening test for *M. pneumoniae* antibodies. In total, 193 sera from patients with respiratory diseases were measured with PAT (negative: n=31; titres 40-160: n=107; titres 320-640: n=27; titres ≥1280: n=28). The following graph shows the corresponding results obtained with **Mycoplasma pneumoniae-IgG-, IgA-,** and **IgM-ELISA medac**.
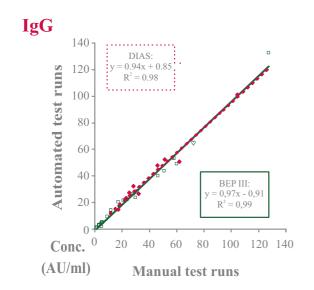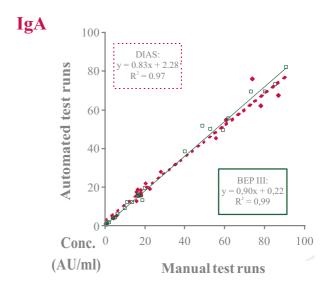

The **Mycoplasma pneumoniae ELISAs medac** provide differentiated results in regard to IgG, IgA, IgM antibodies. Thus, the course of disease can be concretely assessed. By using PAT, differentiation of the three immunoglobulin isotypes is not possible.

Mycoplasma pneumoniae

Automation

The automation of **Mycoplasma pneumoniae-IgG-, IgA-,** and **IgM-ELISA medac** was verified in various open systems/ELISA processors. The concordance between manual and automated test runs is reflected by the very good correlation between the values obtained (see graphs).

IgG

DIAS:
$y = 0.94x + 0.85$
$R^2 = 0.98$

BEP III:
$y = 0,97x - 0,91$
$R^2 = 0,99$

Automated test runs

Conc. (AU/ml)

Manual test runs

IgA

DIAS:
$y = 0.83x + 2.28$
$R^2 = 0.97$

BEP III:
$y = 0,90x + 0,22$
$R^2 = 0,99$

Automated test runs

Conc. (AU/ml)

Manual test runs

IgM

DIAS:
$y = 1.02x + 0.03$
$R^2 = 0.99$

BEP III:
$y = 0.98x + 0.01$
$R^2 = 0.99$

Automated test runs

OD

Manual test runs

IgG-, IgA-, IgM-ELISA medac

Precision

To establish intra-assay and interassay variance, each five sera of different reactivity were tested both manually and with DSX.

For assessment of intra-assay variance these sera were each tested in a 22-fold assay. For assessment of interassay variance these sera were each tested by 11 independent test procedures.

Intraassay variance

Serum	IgG		IgA		IgM	
	AU/ml	VK	AU/ml	VK	OD	VK
1	6	6%	6	3%	0,021	18%
2	16	4%	12	6%	0,542	3%
3	36	5%	21	3%	0,792	6%
4	50	6%	27	2%	0,990	4%
5	88	5%	116	3%	1,480	4%

Interassay variance

Serum	IgG		IgA		IgM	
	AU/ml	VK	AU/ml	VK	OD	VK
1	3	4%	6	3%	0,033	11%
2	34	5%	21	6%	0,651	2%
3	69	5%	22	5%	0,838	2%
4	82	5%	115	10%	1,141	2%
5	128	7%	126	5%	1,611	2%

Mycoplasma pneumoniae

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IgG-, IgA-, IgM-ELISA medac

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Mycoplasma pneumoniae

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