Chlamydia serology: The value of antibody determinations against recombinant chlamydia specific lipopolysaccharide (rLPS) in respiratory infections

Karen Dreesbach, medac GmbH, Diagnostic Division, Wedel, Germany

Introduction
The chlamydiae are a diverse group of bacteria of eubacterial origin. Because of their obligate intracellular reproduction for which they need the host cell’s energy equivalent ATP, they are also called energy parasites. Chlamydiae infect a wide range of hosts and cause a broad range of disease entities.

Taxonomy
The order chlamydiales has one family, chlamydiaceae, and one genus, chlamydia. Currently four species are recognized, Chlamydia trachomatis, Chlamydia pneumoniae, Chlamydia psittaci, and Chlamydia pecorum. To date, 19 different Chlamydia trachomatis serovars (41) and several Chlamydia psittaci serovars are known (6 avian (65), 9 mammalian (29)), but only one Chlamydia pneumoniae (40,74) and one Chlamydia pecorum serovar (15).

Host range and clinical pictures of chlamydia infections
Chlamydia trachomatis and Chlamydia pneumoniae are obligate pathogens of humans (the isolation of Chlamydia pneumoniae from a horse had been reported (75)). This pathogen also was identified in koala bears by OMP2 gene sequencing (20). Chlamydia psittaci is pathogenic for a variety of bird and animal species (12,28), from which it is transmitted to humans (10). To date, Chlamydia pecorum has only been isolated from sheep, cattle, pigs, and koala bears (3,20).

Chlamydia trachomatis causes the endemic blinding trachoma (42) and is one of the most frequent causes of sexually transmitted diseases (18) and their sequelae, among them reactive arthritis (59) and infertility (43).

Chlamydia psittaci infections in birds and animals lead to keratoconjunctivitis, pneumonia, enteritis, polyarthritis, encephalomyelitis, and abortion (7,56). They cause a zoonotic psittacosis in humans (7).

Chlamydia pecorum, which is strictly an animal pathogen, is responsible for conjunctivitis, pneumonia, urinary tract diseases, and abortion (16).

Infections with Chlamydia pneumoniae occur worldwide and have been associated with both endemic and epidemic outbreaks (22,36). Sero-epidemiological studies have shown a mean IgG antibody prevalence of 50% in middle-aged adults throughout the world (51,72). This pathogen has been regarded as one of the most frequent causes of acute and chronic infections of the upper and lower respiratory tract (22,54).

The spectrum of diseases, in addition to flu-like illness, includes sinusitis, pharyngitis, bronchitis, chronic obstructive pulmonary diseases, pneumonia and reactive arthritis (21,36 67). A causal involvement of Chlamydia pneumoniae in asthma (26,27), sarcoidosis (47), lung cancer (32,35), atherosclerosis (14,76), acute myocardial infarction (50,51), brain stroke (13,75), multiple sclerosis (19,60), and late-onset of Alzheimer’s
disease (4), remains a controversial area of investigation; concrete, definitive proof is still lacking.

**Diagnosis of Chlamydia pneumoniae infections**

The laboratory diagnosis of Chlamydia pneumoniae infections includes direct methods and indirect methods. The direct methods are based on isolation of the pathogen from cell culture as well as non-culture techniques, which detect either the whole organism or parts of it (antigen, nucleic acids). The indirect methods detect antibody responses against the causative pathogen. Unlike Chlamydia trachomatis, the diagnosis of Chlamydia pneumoniae infections by culture and nonculture methods, respectively, has, for a variety of reasons, so far proven to be unsatisfactory (53,68).

**Direct methods**

**Culture Isolation**

Isolation from culture is still not a simple and effective method, in spite of improvement using HL or Hep-2 cell lines. With the exception of two laboratories, the isolation rate is very low (53). Several reasons have been discussed, such as transport and temperature conditions (33,38,53,62), considerable variations of growing ability of different strains circulating in different populations (5), paucity of agent in superficial mucous epithelia (53).

**Antigen detection EIA and DFA**

Direct antigen detection by enzyme immunoassays (EIA) or direct fluorescence assays (DFA) has not been widely used. EIA methods initially developed for the detection of Chlamydia trachomatis, measure lipopolysaccharide (LPS), common to all chlamydiae. Theoretically they may be used for the diagnosis of Chlamydia pneumoniae infection (53). However, the low level of Chlamydia pneumoniae LPS in respiratory samples and the possibility of cross-reactions with gram-negative bacterial flora may compromise the use of the assays (66). DFA methods, using species-specific monoclonal antibodies, were reported to be of minor sensitivity (24,48) and also specificity, the latter due to the subjective reading (66).

**Nucleic Acid Amplification**

Nucleic acid amplification has been the most important recent advance in the field of Chlamydia pneumoniae diagnosis. Compared to cell culture, this method is more sensitive and highly specific. Strict quality control is necessary, to avoid false positive results due to DNA contamination (34); false negative results may occur due to the presence of PCR inhibitors in respiratory specimens. False negative results are difficult to correct since they occur unpredictably and are only detectable, if amplification controls, such as a PCR for human β-globin or spiking duplicate samples with chlamydial DNA, are routinely used. To date, a commercially available, standardized test has yet to be developed yet, which delays its implementation for Chlamydia pneumoniae on a routine basis in a clinical laboratory (66).

For all these tests the true sensitivity of the results may be reduced, if the samples are not directly taken from the diseased organ (53); the same is valid for the specificity of results, because asymptomatic, non-pathogenic carriage of Chlamydia pneumoniae in the upper respiratory tract seems to be a common phenomenon (67).
**Indirect methods**
The shortcomings of culture and non-culture methods for the diagnosis of Chlamydia pneumoniae infections have made serology the method of choice. The antibodies detected are directed towards the lipopolysaccharide (LPS) and the major outer membrane protein (MOMP) (Fig.1).

![Indirect Methods](image)

Fig.1: Main antigens of the outer membrane complex of Chlamydia pneumoniae

The LPS antigen is genus specific. The MOMP is the most prominent protein of the outer membrane complex of chlamydial elementary bodies, which comprises approximately 50% of all proteins in the outer membrane (11). It has subspecies, species, and genus specific epitopes (2).

A variety of serological tests have been used. Currently the most widely employed assays are the rLPS-ELISA (rELISA), and microimmunofluorescence (MIF); the complement fixation test (CFT) is still used by some laboratories. More recently a variety of species specific enzyme immunoassays (EIA) have been developed and released commercially.

**CFT**
CFT has traditionally been used for the diagnosis of Chlamydia psittaci infections (49); it employs Chlamydia psittaci LPS as antigen, which contains the genus specific epitopes, that are found in all chlamydia species. It cannot discriminate between antibody isotypes. The CFT is still of value for the diagnosis of ornithosis and lymphogranulomatosis venereum (61). In respiratory infections the CFT is sensitive only in primary infections of young adults. In children and adults with primary infections and reinfections, respectively, up to 90% of Chlamydia pneumoniae infections will not be identified by CFT(53).
**Species-specific EIA**

More recently a variety of species specific EIA methods have been developed and released commercially. These can be divided into two groups according to the antigen preparations used:

Group 1 uses purified total elementary bodies of *Chlamydia pneumoniae* or purified membrane fragments, both still with LPS. In this group species specificity can be excluded.

Group 2 employs total elementary bodies or purified membrane fragments, which have been LPS absorbed. Also here species specificity is questionable for two reasons:
1. Internal investigations with chlamydial LPS-specific monoclonal antibodies have shown, that in spite of LPS absorption or degradation still a considerable amount of this antigen remains in the preparations (9).
2. These, partly LPS absorbed, distorted elementary bodies expose cross-reactive epitopes common to all chlamydia and tissue-culture derived contaminants may react with tissue autoantibodies (53).

Extensive investigations of these tests on a broad population basis with various clinical cohorts are still needed, in order to see, how they actually perform, especially as regards species specificity.

**MIF**

The MIF of Wang and Grayston was developed in the early 70s for the diagnosis of *Chlamydia trachomatis* infections (70,71). After the discovery of *Chlamydia pneumoniae* it was applied to its diagnosis, and considered as the standard for identifying *Chlamydia pneumoniae* infections (73).

The inherent technical problems of MIF are well known:
- The test is laborious, highly trained technicians are required to distinguish between specific (MOMP), genus specific, cross-reactive (LPS), and non-specific fluorescence patterns; and even then the reading is very subjective (1,55,57).
- MIF is not standardized; the use of different antigens (76) and different cut off criteria for past and recent or current infections (6,25,30,46,50) creates significant lab-to-lab variations of results (Table 1).

---

**Microimmunofluorescence (MIF)**

Serological Criteria Used by Different Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Chronic Infections</th>
<th>Acute Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saikku et al.</td>
<td>IgG &gt; 1:256, IgA &gt; 1:16</td>
<td>4-fold or greater titer increase for any Ig class or IgG &gt; 1:512, IgA &gt; 1:512, or IgM &gt; 1:16</td>
</tr>
<tr>
<td>Puolakkainen et al.</td>
<td>IgG &gt; 1:16, IgA &gt; 1:8</td>
<td>IgG &gt; 1:512, IgM &gt; 1:16 or seroconversion</td>
</tr>
<tr>
<td>Thom et al.</td>
<td>IgG &gt; 1:16, IgA &gt; 1:8</td>
<td>IgG &gt; 1:512, IgA &gt; 1:256</td>
</tr>
<tr>
<td>Blasi et al.</td>
<td>IgG &gt; 1:16, IgA &gt; 1:8</td>
<td>IgG &gt; 1:512, IgA &gt; 1:256</td>
</tr>
<tr>
<td>Gurfinkel</td>
<td>IgG &gt; 1:128, IgA &gt; 1:8</td>
<td>IgG &gt; 1:512 or 4-fold increase in IgG or IgA</td>
</tr>
<tr>
<td>Gieffers</td>
<td>IgG &gt; 1:128, IgA &gt; 1:8</td>
<td>IgG &gt; 1:512 or 4-fold increase in IgG or IgA</td>
</tr>
<tr>
<td>del Piano et al.</td>
<td>IgG &gt; 1:32, IgA &gt; 1:8</td>
<td>IgG &gt; 1:512 or 4-fold increase in IgG or IgA</td>
</tr>
</tbody>
</table>

Table 1: Examples of different cut off criteria in MIF leading to different results
The diagnosis of primary infections by MIF is faced with the problem, that MOMP antibodies appear relatively late after onset of disease, IgM about 3 weeks later, IgG about 8 weeks later. Complement fixing antibodies are already detectable in the acute serum. In reinfection there is often no CF antibody and no IgM antibody response. The IgG antibody response may appear within three weeks and may rise to titers >512 (22) (Table 2).

The diagnosis of recent infections by demonstrating fourfold titer changes of IgG antibodies, or the diagnosis of acute infections by the detection of IgM antibody titers ≥ 16 or IgM seroconversion, is well accepted (23). As IgM antibodies are normally only detectable in primary infections and as fourfold IgG-titer changes cannot be observed regularly, single IgG titers ≥ 512 had been defined as indicators of current infections (24). However, this had been arbitrarily set and never evaluated on a broad population basis (58). Critics of the use of a single antibody titer to diagnose current infection, refer to the subjective reading of the results, which may lead to differences up to two titer stages, and to the relatively long persistence, even of high antibody titers, after infection (17,31,45).

In more recent years the value of the MIF also has been disputed as regards species specificity versus cross-reactivity (1,31,77).

To summarize the features of the MIF:

- The delayed antibody response in primary infections may lead to false negative results if blood had been collected too early.
- Early proper antibiotic treatment can prevent antibody formation (30).
- The persistence of antibody may cause problems in diagnosing recent or current infections. If only results from single sera are available or if no titer movement can be observed in paired sera, the discrimination between past or current chlamydia exposure is limited.
- On the other hand, this persistence enables seroepidemiological studies, to which the MIF rigorously had been applied, and, to date, has been considered as the method of choice (57).
rLPS-ELISA
Some years ago test systems had been postulated, which should fulfill the following criteria:
• Use of defined antigens and concordant detection of defined antibodies.
• Objective, reproducible, standardized results.
• Rapid, quantitative, automatable, use for large/small routine diagnosis.
• Possibility to discriminate between past and current chlamydia exposure.
• Possibility of early diagnosis/early treatment.
• Possibility of post-treatment follow-up.
• High sensitivity.
• Species specificity.

The first EIA, which has considered this wish list, is the rLPS-ELISA (rELISA) for the detection of IgG, IgA and IgM antibodies. The rELISA was developed in the beginning of the 90s after extensive basic research (8). It employs a chemically pure fragment of the chlamydial LPS that has been defined with the use of chemical and molecular biology techniques (Fig.2).

It is produced by recombinant technology and contains two chlamydia-specific epitopes (C,D; Fig.2) and two epitopes, which are shared between chlamydial LPS and Salmonella minnesota RE-type LPS (A,B; Fig.2). This crossreactivity does not influence the clinical diagnosis, as this RE-type is an artificial laboratory strain (8).

The technical criteria of defined antigen, standardization, reproducibility, objectivity, suitability for small- and large-scale routine diagnosis, and automation are fulfilled. Additionally end titers can be determined from the starting dilution without endpoint titrations.

In the following some clinical-diagnostic examples are presented which demonstrate the significance of LPS antibody determinations.
**Reproducibility**
The intra-assay variability of the Chlamydia IgG, IgA, IgM rELISA was calculated by testing 4 positive sera 21 times in 1 test run. The inter-assay variability was calculated by testing 4 positive sera in 11 different test runs (Fig.3).

- The intra-assay variability ranged from 4.02 to 5.75%.
- The inter-assay variability ranged from 7.13 to 10.00% (40).

![Chlamydia rELISA Reproducibility](image)

**Diagnostic criteria**
The criteria for significant titer increases for diagnosis of current chlamydia-specific infections have been independently established by Verkooyen et al. (67) and Persson et al. (45).

Verkooyen has employed a reference panel of sera from patients with respiratory diseases, showing a significant titer increase in MIF.

Persson has used a reference panel of sera with significant titer increases in CFT.

The corresponding rELISA results led to the following diagnostic criteria:

- threefold or greater increase in specific IgG or IgA antibody titer
- twofold or greater increase in specific IgG antibody titer in combination with twofold or greater increase in specific IgA antibody titer
- twofold or greater increase in specific IgM antibody titer
Identification of current infection (68)
N=1104 healthy blood donors and n=271 patients with chronic obstructive pulmonary disease (COPD) had been tested for the prevalence of chlamydia-specific antibodies by rELISA and MIF (Fig.4).

![Chlamydia rELISA Discrimination between Past and Current Infections](image)

Fig.4: Comparison of MIF and rELISA for identification of current chlamydial infections

The prevalence in the healthy blood donor group and the COPD patient group was 29.4% and 53.1% respectively, as determined by rELISA. The difference was highly significant, p<0.0001. The seroprevalence by MIF was with 71% and 72% the same for healthy blood donors and COPD patients, respectively. The different rELISA seroprevalence in the two groups indicates, that the half-life of chlamydial LPS antibodies seems to be much lower as compared to MOMP antibodies. Since LPS is a T-cell independent antigen, the antibodies do not persist as long as antibodies directed towards proteins, which are T-cell dependent antigens. The rELISA allowed good discrimination between current infection and obviously past contamination, which was not the case with MIF.

LPS serology in patients with community acquired pneumonia (CAP) (66)
Paired sera from n=156 patients with community acquired pneumoniae were enrolled by MIF, rELISA, and PCR. The diagnostic criteria for rELISA have already been mentioned earlier. The diagnostic criteria for MIF based on a fourfold or greater titer increase in Chlamydia pneumoniae specific IgG and/or IgA antibodies (Fig.5).
The diagnosis of acute Chlamydia pneumoniae pneumonia was established in n=45 patients (29%) by any of the test systems. These patients were divided into two groups: sero-responders and sero-non-responders. 23 Patients (51%) were defined as sero-responders, because they met the diagnostic criteria of acute Chlamydia pneumoniae infection. 9 of these patients were additionally PCR positive (39%). 22 patients (49%) were PCR positive without serological evidence of acute Chlamydia pneumoniae infection; they were either seronegative or had constant antibody titers.

15/23 sero-responders (65%) had MIF results indicative for acute Chlamydia pneumon-ia CAP, and 20/23 (87%) had corresponding rELISA results. Significantly more Chlamydia pneumoniae infections were confirmed by PCR in those patients, who already had specific LPS antibodies at admission (38% PCR positive) as compared with the seronegative ones (21% PCR positive), p=0.021. This difference was not observed, when the MIF was used to discriminate between sero-negative and sero-positive patients (30% PCR positive and 27% PCR positive, respectively), p=0.842. This may be explained by a more rapid appearance of chlamydial LPS antibodies in acute infection as compared to that of MOMP antibodies, detected by MIF. Certainly, these numbers are too small for a definite conclusion, and should be extended.
LPS serology in recent infections in children (64)

N=224 children, age 6 months to 20 years, whose clinical data and patient records indicated the presence of a respiratory tract infection (RTI), were enrolled. A lower RTI was defined, if there were auscultatory findings of involvement of the lower respiratory tract and/or X-ray abnormalities. The control group consisted of 69 patients without respiratory complaints and was matched according to the same criteria as the RTI patients. For antibody detection CFT, MIF, and rELISA were employed.

There was no correlation between the percentage of positive tests and patient characteristics like gender, the presence of either upper or lower respiratory tract infections or the status inpatient/outpatient (Fig.6).

The patients had been divided into different age groups of 0-5 years, 6-10 years, 11-15 years, and 16-20 years (Fig.7). The percentages of positive tests increased with the different age groups significantly, and were 4.7%, 9.5%, 13.3%, and 17.4%, respectively. The particularly high increase between children not yet attending school (0-5 years) and school attending children may be explained by the fact, that the last group is at increased risk for contact with Chlamydia positive children.

Based on overall test results (Fig.8) chlamydial RTI was caused in 44 patients, which corresponds to 19.6%. Only 2 patients (0.8%) showed a positive CFT response, 19 patients (8.5%) were positive in MIF and 34 patients (15.2%) positive in rELISA. Only one patient was MIF-positive in the control group, it was the same patient who was also positive for LPS antibodies.
The rELISA positive/MIF negative results were further analyzed in order to see, if the lower sensitivity of the MIF could be explained by different antibody kinetics as regards the production of measurable LPS and MOMP antibodies after onset of disease.

Sera from patients with RTI were divided into two groups (Fig.9):
Group 1 consisted of \( n = 99 \) sera collected within two weeks after onset of disease.
Group 2 consisted of \( n = 86 \) sera collected later than two weeks after onset of disease.

Fig.8: Value of different serological tests for diagnosis of Chlamydia pneumoniae RTI in children

Fig.9: Kinetics of LPS and MOMP antibodies in relation with time of blood collection
The later appearance of measurable MOMP antibodies was illustrated by the higher percentage of positive MIF results in sera collected later than two weeks after onset of disease than within two weeks (13% versus 5%). This difference was not seen in rELISA, with which 15% of the sera were positive within two weeks after onset of disease, a rate that stayed stable later than two weeks after onset of disease. These findings are in agreement with the previously mentioned experiences of Grayston et al. and Saikku (22,53).

**Chlamydia LPS and MOMP antibody kinetics in patients at different stages of upper and lower respiratory tract infections**

In the following some examples are presented which demonstrate the LPS and MOMP antibody kinetics at different stages of upper and lower respiratory tract infections. Furthermore the examples provide an idea on the individually different immune responses.

Most of the 165 patients tested had three consecutive sera collected.

**Tables 2-6: Comparison of LPS and MOMP antibody kinetics by rELISA and MIF in patients with respiratory tract infections and atypical pneumonia**

- Patient X (Table 3) already showed a clear LPS antibody titer of 400, 5 days after onset of disease, which increased eightfold within 9 days. 47 days after onset of disease a sixteen-fold increase was monitored. A further 48 days later a sharp IgG decrease had taken place. IgA was initially positive with a fourfold titer increase 47 days later. In the MIF a clear seroconversion of MOMP IgM antibodies 14 days after onset of disease was observed with a concomitant seroconversion of LPS.
IgM. The patient stayed MIF IgG negative until day 95, at which a seroconversion towards a titer of 16 was shown.

- Patient Y (Table 4) seroconverted for LPS IgM antibodies 10 days after onset of disease with a subsequent fourfold titer increase after another 10 days. At that time he also seroconverted for LPS IgG and IgA antibodies. The MOMP IgM titer movement was in parallel with the LPS IgM movement; however, this patient stayed negative for MOMP IgG antibodies over the whole observation period. This fits again into the observations of Grayston et al. and Saikku as regards the delayed MOMP IgG antibody response in primary infections (22,53).

- The first serum of patient 1419 (Table 5) was LPS IgG positive with an eightfold titer increase, demonstrated 12 days later. At that time he had seroconverted for LPS IgA antibodies. He had an initial MOMP IgG antibody titer of 512, which did not change over the time (44).

- Patient 1706 (Table 6) was positive in the first serum for all antibody isotypes by both rELISA and MIF. The MIF demonstrated basic antibody titers of 512 and 256, respectively, which did not change over the observation period of 64 days. rELISA revealed extremely high LPS antibody titers already in the first serum with an additional sixfold IgA increase after 14 days (44).

Tables 7-10: Comparison of LPS and MOMP antibody kinetics by rELISA and MIF in patients with respiratory tract infections with and without treatment

- Patient 1506 (Table 7) was negative for both LPS and MOMP IgM antibodies. The initial MOMP IgG and IgA antibody titers of 256 remained constant over the pe-
period of 15 days. Within this time he had had a striking seroconversion of LPS-IgA antibodies as well as an eighteen-fold IgG titer increase (44).

- Patient 1617 (Table 8) remained constantly MOMP IgG and IgA positive with negative IgM. The initially already high LPS IgG and IgA antibody titers had increased ninefold and twelve-fold, respectively, after 12 days. At that time he had clearly seroconverted for LPS IgM. All antibody isotypes remained at a constant level until day 47 (44).

- The patient from Table 9 anamnestically already had two defined Chlamydia pneumoniae infections, the first expressed in form of a mild, flu-like illness, and the second in form of an obstructive bronchopneumonia. Shortly after both infections she had become seronegative for LPS IgM and IgA antibodies, with a remaining low IgG. Six months before she presented with an acute cervical lymphadenitis, the LPS antibody status was 100 for IgG and negative for IgA and IgM. Two days after onset of symptoms the patient showed a fourfold LPS IgG titer increase, as well as seroconversion for IgM and IgA. The MOMP IgG and IgA titer were 64, and IgM was negative. Chlamydia pneumoniae specific PCR and direct fluorescence assay in gargle wash were positive. The patient received azithromycin orally at a dose of 500 mg/day for three days. Eight days after the end of treatment LPS IgM and IgA serology gave negative results, MOMP IgA showed a twofold titer decrease. PCR and DFA were negative. Another eight days later the LPS antibody status remained unchanged, MOMP IgA demonstrated again a twofold titer decrease, but still remained positive (40).

- The patient presented in Table 10 with acute respiratory disease initially had high LPS IgG antibodies and a positive IgA. Ten days later the IgG titer had increased threefold, IgA almost twofold. The patient was treated for 21 days with 200 mg doxycycline/day orally. On day 39 he was negative for LPS IgA, accompanied by a two and a half fold IgG titer decrease (40). Over the whole observation period he remained negative for all MOMP antibody isotypes. This may serve as an example for early diagnosis of a primary infection (no MOMP antibody seroscan) by LPS serology with subsequent early, appropriate treatment which prevents MOMP antibody formation.

- The patient with respiratory disease (Table 11) and low basic LPS antibody titers had become negative for the low titered IgM already during the 14-days treatment with 200 mg doxycycline/day. Another 22 days later IgG was no more detectable. IgA still remained at a low titer. MOMP IgG and IgA titers did not change over the observation period (40).

- The patient presenting with atypical pneumonia (Table 12) was basically negative for all LPS antibody isotypes, and positive for MOMP IgG and IgA. 10 days later he strongly seroconverted for all LPS antibody isotypes, as well as for MOMP IgM. After a 14-days treatment course with 200 mg doxycycline/day, on day 36 a fivefold IgA titer decrease and an almost twofold IgM decrease were observed, while IgG still had further increased. The MOMP antibody titers remained unchanged (40).
In summary, the LPS antibody titer kinetics may be of help in early diagnosis of acute or recent infections and post-treatment follow up, which could not be demonstrated by MOMP antibodies.

LPS antibody seroconversion can be detected within five days, but can be missed, if paired blood samples are collected too late (30). LPS antibody titer increases, indicating to an acute or recent infection, can be observed, if blood is collected within two to three weeks after onset of symptoms. If blood is collected later than two to three weeks after onset of disease, LPS antibody titer increases may already be missed, because of the rapid LPS antibody dynamics (37). In this event the results would show stable antibody levels, which may be an indication of an already chronic manifestation.

It can also happen, that LPS antibody titers, especially short-lived IgA, remain stable after appropriate treatment, even if the symptoms have disappeared. Chronic, silently persisting infections have been discussed, in which continuous antigen shedding occurs, that may act as a chronic stimulus for IgA production (50). In these cases the patient should be controlled every six to twelve months for antibody titer changes.
Genus specific chlamydia LPS serology for species specific diagnosis of Chlamydia pneumoniae infections (69)

To evaluate, if genus specific LPS serology can be employed for specific diagnosis of Chlamydia pneumoniae infections, each 3 consecutive sera from 22 COPD patients with significant LPS titer increases, indicating acute chlamydia infections, were selected; they were retested with a commercially available MIF, which discriminates between Chlamydia pneumoniae, Chlamydia trachomatis, and Chlamydia psittaci (68). They were also investigated with a recently developed Chlamydia trachomatis specific, peptide based ELISA (pELISA medac). A titer increase would have been expected in this assay, if there had been a current Chlamydia trachomatis infection interfering with Chlamydia pneumoniae diagnosis (69) (Fig.10).

Fig.10: Investigation on interference of Chlamydia trachomatis antibodies with specific Chlamydia pneumoniae diagnosis by LPS serology

Only two patients had positive Chlamydia trachomatis IgG antibody responses; however, they remained at a stable level in the consecutive sera, indicating past exposure. All patients were negative for IgA antibodies.

With both MIF and pELISA no evidence indicating current Chlamydia trachomatis or Chlamydia psittaci infection was found (67,69).

Summary

The rELISA meets, for the most part, the clinical-diagnostic postulations, such as early diagnosis of acute and recent infections, corresponding post-treatment follow-up and discrimination between past and current infections, respectively. In chronic infections LPS IgG and IgA antibodies seem to reflect actively ongoing or silently persisting processes, indicated by antibody persistence more than 6 months after appropriate treatment. However, this is still under discussion and has to be further evaluated. As shown, the rELISA is not suitable for diagnosing past infections; for these epidemiological questions MIF is method of choice.
Despite the genus-specific status of the rELISA it has been said to be the currently most sensitive, commercially available serological method for the diagnosis of acute or recent Chlamydia pneumoniae infection (66,67,68).

Acknowledgement
- I thank Prof. Gunna Christiansen and Dr. Svend Birkelund, University of Aarhus, Denmark, for the kind permission, to use their figure of the Chlamydia pneumoniae membrane structure.
- I am grateful to Prof. Helmut Brade, Research Center for Experimental Biology and Medicine, Borstel, Germany, for the figure showing the chemical structure of the chlamydia-specific LPS-fragment.
- I value the independent investigations on chlamydia LPS serology by
  - Dr. Kenneth Persson, University of Lund, Sweden
  - Dr. Jeroen Tjhie, Academical Hospital Maastricht, The Netherlands
  - Dr. Roel Verkooyen, Erasmus University, Rotterdam, The Netherlands
References


43. Patton DL, Askienazy-Elbhar M, Henry-Suchet J, Campbell LA, Cappucio A, Tan-
nous W, Wang SP, Kuo CC: Detection of Chlamydia trachomatis in fallopian tube
171:95-101


45. Persson K, Haidl S: Evaluation of a commercial test for antibodies to the chlamy-
dial lipopolysaccharide (Medac™) for serodiagnosis of acute infections by Chlamydia pneumoniae (TWAR) and Chlamydia psittaci. APMIS (2000) 108:131-
138

response to Chlamydia pneumoniae in adults with coronary arterial fatty streaks

47. Puolakkainen M, Campbell LA, Kuo CC, Leinonen M, Gronhagen-Riska C, Saikku
P: Serological response to Chlamydia pneumoniae in patients with sarcoidosis. J
Infect (1996) 33:199-205

48. Roblin PM, Dumomay W, Hammerschlag MR: Use of Hep-2 cells for improved iso-
1971


JK, Valtonen V: Serological evidence of an association of a novel chlamydia,
TWAR, with chronic coronary heart disease and acute myocardial infarction.

51. Saikku P, Leinonen M, Tenkanen L, Linnanmäki E, Ekman MR, Manninen V,
Mänttäri MD, Frick H, Huttunen JK: Chronic Chlamydia pneumoniae infection as a
risk factor for coronary heart disease in the Helsinki Heart Study. Ann Intern Med
(1992) 116:273-278

52. Saikku P: The epidemiology and significance of Chlamydia pneumoniae. J Infect

53. Saikku P: Diagnosis of acute and chronic Chlamydia pneumoniae infections. In:
Orfila J, Byme GI, Chernesky M et al., eds. Proc 8th Int Symp on Human Chlamydial
Infections (1994):163-172

54. Saikku P: Chlamydia pneumoniae – clinical spectrum. In: Stephens RS, Byme GI,
Christiansen G et al., eds. Proc 9th Int Symp on Human Chlamydial Infections

55. Schachter J: Human Chlamydia psittaci infection. In: Oriel D, Ridgway GR,
Schachter J et al., eds. Proc 6th Int Symp on Human Chlamydial Infections
(1986):311-320

Date of issue: June 2000


