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Comparison of Serological Assays for Detection of *Chlamydia trachomatis* Antibodies in Different Groups of Obstetrical and Gynecological Patients

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New serological enzyme immunoassays (EIAs) were compared with microimmunofluorescence (MIF) as a "gold standard" to detect *Chlamydia trachomatis* antibodies in different groups of obstetrical, gynecological, and subfertile patients. There were no significant differences in seroprevalence rates, except for the group of *C. trachomatis*-positive patients ($P < 0.01$). Test characteristics were calculated for Chlamydia-EIA (Biologische Analysensystem GmbH, Lich, Germany) and pELISA (Medac, Wedel, Germany). pELISA seems to be a good alternative to MIF. It has high specificity and is easier to perform.

Recently new commercially available species-specific (peptide-based) enzyme immunoassays (EIAs) have been developed for the detection of *Chlamydia trachomatis* antibodies. So far they have missed clinical evaluation. Serological assays have been used to detect antichlamydial antibodies in the fertility workup (10), predicting tubal pathology (5, 7). Their value for fertility evaluation remains the subject of debate. There is wide variation between various tests in the correlation of antichlamydial antibodies with current *C. trachomatis* infections or tubal pathology. The species-specific microimmunofluorescence assay (MIF) is considered to be the "gold standard" for the serological diagnosis of *C. trachomatis* infections (12). Cross-reactivity with *Chlamydia pneumoniae* in the existing assays should be taken into account (3). MIF is laborious, and reading of the assay is subjective, and therefore it is not suitable for a daily routine. EIAs provide objective reading and allow the handling of more samples at the same time. We compared two new serological assays with MIF to determine the performance of these assays in the routine serodiagnosis of *C. trachomatis* infections.

For our serological studies, we divided sera from obstetrical and gynecological outpatients into four different groups: subfertility patients ($n = 76$), pregnant women ($n = 150$), a control group that includes a randomly selected group of women who visited our outpatient department with various complaints unrelated to subfertility or pregnancy ($n = 220$), and women found positive for *C. trachomatis* in a direct antigen assay ($n = 40$). Some women in the last group were also represented in the subfertility or pregnant group ($n = 2$ and $n = 5$, respectively).

For serological diagnosis, we used two EIAs. The CT-pELISA (Medac, Wedel, Germany) was used to perform species-specific serology by using a synthetic peptide from the immunodominant region of the major outer membrane protein. This highly specific antigen makes it possible to discriminate between *C. trachomatis*-specific antibodies and the whole anti-*Chlamydia* antibody response.

The BAG-Chlamydia-EIA (Biologische Analysensystem GmbH, Lich, Germany) uses the ultrasonicated whole-cell *C. trachomatis* antigen (strain LGV type 17). If *C. trachomatis* antibodies are present in the specimen, they will react with the antigen. Both microtiter assays use peroxidase-conjugated antihuman immunoglobulin G (IgG) and IgA antibodies to bind to *C. trachomatis* IgG and IgA antibodies. After incubation with tetramethylbenzidine substrate, the reaction is stopped by the addition of sulfuric acid. The absorption is read photometrically at 450 nm. The intensity of the color is proportional to the concentration (or titer) of the specific antibody in the sample.

Cutoff values were calculated according to the manufacturers' instructions. Results in the gray zone were considered negative in the calculations.

An indirect MIF antibody technique was used as a gold standard to detect *C. trachomatis* IgG antibodies (egg-grown *C. trachomatis* biovar L_2; BioMérieux, Hertogenbosch, The Netherlands). Sera were diluted to a titer of 1:64 in phosphate-buffered saline (PBS). After incubation and washing in PBS, a conjugate (Fluoline-G; Evans blue diluted in PBS) was added to the samples. After 30 min of incubation at 37°C and being washed in PBS, the slide was covered with a coverslip with mounting medium. A fluorescence microscope was used for the reading of the slides. A positive reaction is represented by a "starry sky" appearance: fluorescent green spots on a slightly red background. Two experienced persons evaluated all samples. When discrepancies occurred, a third person evaluated the sample.

For comparison of the EIAs to the MIF assay and to detect tubal pathology, two-by-two tables were used to calculate sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The chi-square test was used to

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TABLE 1. Prevalence of *C. trachomatis* antibodies (IgG and IgA) in different gynecological patient groups according to different assays

Group (*n*)	Prevalence[a]		
	Chlamydia-EIA	pELISA	MIF(IgG)[b]
Subfertility (76)			
IgG	31.6 (24)	21.1 (16)	31.6 (24)
IgA	2.6 (2)	1.3 (1)	
Pregnant (150)			
IgG	24.7 (37)	17.3 (26)	23.3 (35)
IgA	2 (3)	3.3 (5)	
Control (220)			
IgG	31.4 (69)	19.1 (42)	31.4 (69)
IgA	3.6 (8)	5 (11)	
C. trachomatis positive (40)			
IgG	82.5 (33)	65 (26)	62.5 (25)
IgA	12.5 (5)	17.5 (7)	

[a] Values are percentages. Each parenthetical value is *n*.
[b] Sera diluted to a titer of 1:64.

test the significance of the difference in frequency distribution. A *P* value of <0.05 was considered significant.

The seroprevalence rates in the subfertility, pregnant, and control groups are described in Table 1. No significant differences in overall prevalence rates of *C. trachomatis* IgG antibodies were found in all three assays. The prevalence of *C. trachomatis* IgA antibodies is very low. Significantly higher prevalences of *C. trachomatis* IgG antibodies were found in the group of *C. trachomatis*-positive patients ($P < 0.01$) (Table 1). Also, a significant ($P < 0.05$) increase in the prevalence of *C. trachomatis* IgA antibodies was found. The Chlamydia-EIA has a good correlation with the prevalence of *C. trachomatis* IgG antibodies. This assay detected the highest percentage of *C. trachomatis*-positive patients (82.5%). The test characteristics of the two EIAs are described in Table 2.

Tubal patency is essential for fertility. Patency is tested either by visualizing the tubes by X ray with contrast fluid (hysterosalpingography) or by direct observation by laparoscopy during which the tubes are pertubated. In 32 subfertility patients, tubal patency was tested. Twenty-one patients had patent tubes. In 14 of these patients, no *C. trachomatis* IgG antibodies were found (67%). In 11 patients, tubal pathology was found. However, in three patients, none of the assays showed the presence of *C. trachomatis* IgG antibodies (27%). There was a significant difference in serology in the MIF assay between the patients with tubal pathology versus those without ($P < 0.02$). Table 3 shows the test characteristics of the three assays as predictors of tubal pathology.

TABLE 3. Test characteristics of the Chlamydia-EIA, pELISA, and MIF in relation to tubal pathology

Parameter[a]	Result		
	Chlamydia-EIA	pELISA	MIF (IgG)
Sensitivity	54.6	36.4	63.6
Specificity	71.4	85.7	81
PPV	50	57.1	63.6
NPV	75	72	81

[a] For all parameters, values are percentages.

There is little literature available describing performance of new assays. pELISA has been evaluated in infertility patients especially to describe its role in predicting tubal factor infertility (4, 8, 9). Blood samples from healthy female blood donors or pregnant women were commonly used as controls. In our subfertility group, the prevalence rates of *C. trachomatis* IgG and IgA antibodies according to the pELISA and MIF assay were lower than those described in other studies using the pELISA (1, 3, 6, 8, 9). However, in other studies, the titer at which the MIF assay is considered positive is often not mentioned. A lower titer will give more false-positive results. Another reason for the lower prevalence rate in our subfertility group might be that this group includes only a small number of patients with tubal factor infertility (TFI) ($n = 11$), where in other groups, larger numbers of TFI patients were found. Therefore, comparison with non-TFI patients might be more applicable.

The prevalence rates of *C. trachomatis* IgG and IgA antibodies found in our group of pregnant women are in the same range as those found in other studies for the pELISA and slightly higher than those for the MIF assay (1, 8, 9).

Our control group differs from other control groups, since it includes women visiting the Obstetrics and Gynecology Outpatient Department for various complaints. When we compare our group with blood donors and other asymptomatic patients, we find for the pELISA IgG and MIF a higher prevalence rate and for the pELISA IgA a prevalence rate in the same range (1, 6, 9).

Regarding our group of *C. trachomatis*-positive patients, the pELISA shows approximately the same prevalence rates, while with the MIF assay, we found a lower prevalence (1, 6).

We have no explanation for the lower prevalence and sensitivity found with the pELISA in all patient groups. We did

TABLE 2. Test characteristics of the Chlamydia-EIA and pELISA in relation to MIF in different groups[a]

Parameter[b]	Result					
	Subfertility		Pregnant		Control	
	Chlamydia-EIA	pELISA	Chlamydia-EIA	pELISA	Chlamydia-EIA	pELISA
Sensitivity	66.7	58.3	77.1	62.9	76.8	47.8
Specificity	84.6	96.2	91.3	96.5	89.4	94
PPV	66.7	87.5	73	84.6	76.8	78.6
NPV	84.6	83.3	92.9	89.5	89.4	79.8

[a] MIF (IgG) was used as the gold standard.
[b] For all parameters, values are percentages.

not find any study describing the performance of the Chlamydia-EIA.

By using the MIF assay as the gold standard, the test characteristics of the Chlamydia-EIA and pELISA for the determination of serological evidence of a recent or past *C. trachomatis* infection therefore depended on the patient group tested (Table 2). Both tests have reasonably high specificity and NPV and would therefore match the criteria of a screening test. We have to consider that cross-reactivity with other *Chlamydia* species also occurs in the MIF assay (3, 11).

When serology is used to detect tubal pathology, high specificity is important. However, when we used tubal pathology as the gold standard, the specificity and NPV are somewhat lower (Table 3). We have to consider that these results are based on a small number of patients ($n = 32$). In the patients with patent tubes, 33% had *C. trachomatis* IgG antibodies, and in patients with tubal pathology, 27% had no *C. trachomatis* IgG antibodies. Therefore, none of the three assays we used appeared to be perfectly able to predict tubal pathology. Gijsen et al. described no significant differences between two peptide-based EIAs and the MIF in predicting tubal pathology (4). Our test results were comparable, with the exception of the pELISA, which showed a lower sensitivity and higher specificity.

And what about the IgA antibodies? In patients with a current *C. trachomatis* infection, low prevalence rates of IgA antibodies are found (Table 1). It seems that the IgA antibodies can persist for years, even after effective therapy (2). Therefore, IgA antibodies do not indicate a current *C. trachomatis* infection. The role of IgA antibodies in the serodiagnosis of *C. trachomatis* with the currently available assays remains negligible.

pELISA seems to be a good alternative for MIF for the detection of *C. trachomatis* antibodies. pELISA is more species specific than the Chlamydia-EIA. It is less laborious and less expensive than MIF. A screening test needs high specificity and a high NPV. When the two assays are compared with MIF as the gold standard, pELISA has the highest specificity, and in the subfertility group, it has an NPV comparable to that of Chlamydia-EIA.

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