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Chlamydia trachomatis Species Specific Serology: ImmunoComb Chlamydia Bivalent versus Microimmunofluorescence (MIF)

Summary: The ImmunoComb® Chlamydia Bivalent IgG/IgA (Orgenics, Israel) is a new quantitative serologic test that employs LPS extracted *Chlamydia trachomatis* L2 and LPS extracted *Chlamydia pneumoniae* elementary bodies on two separate antigenic spots. The Bivalent *C. trachomatis* specific test results were compared with microimmunofluorescence (MIF), the gold standard of chlamydial species specific serology. For *C. trachomatis* IgG the Bivalent was highly concordant with the MIF: the rate of positive titres (IgG ≥ 1:8) was 10% vs. 11% in 100 blood donors, 18% vs. 16% in 111 obstetric patients (6% antigen prevalence), 26% vs. 22% in sterile women with open (n = 54) and 86% vs. 84% with occluded (n = 51) tubes, and 88% vs. 85% in 103 women with *C. trachomatis* positive cervical smears. Surprisingly, the Bivalent differed considerably from the MIF in IgA prevalence: in obstetric patients (8% vs. 4%), sterile women with open (13% vs. 6%) and occluded (71% vs. 20%) tubes, and women with positive cervical smears (78% vs. 24%). Bivalent IgA appeared to be more sensitive than MIF IgA and showed a stronger correlation with positive cervical smears in obstetric patients (sensitivity 67% vs. 0%, specificity 95% vs. 96%, positive prediction 44% vs. 0%, negative prediction 98% vs. 94%) and with tubal occlusion in sterile women (sensitivity 71% vs. 20%, specificity 87% vs. 94%, positive prediction 84% vs. 77%, negative prediction 76% vs. 55%). MIF IgM was of little diagnostic help. Supplemental to the often difficult *C. trachomatis* antigen detection, the easily performed Bivalent IgG/IgA appears to be of great value in routine diagnosis of genital chlamydial infections.

Introduction

Chlamydia trachomatis is the leading sexually transmitted bacterial pathogen in western industrialized societies [1] with a prevalence rate of 10–15% in older teenagers, rapidly declining after the age of 25–30 years [2]. *C. trachomatis* is estimated to be responsible for two-thirds of tubal factor sterility [3]. About 10% of all couples in industrialized countries are unwillingly childless with about one-half of these cases caused by sterility of the female partner. Tubal occlusion is responsible for about one-third of female sterility. According to this rough estimate, about 1% of all women of reproductive age suffer from sterility as sequelae of a genital chlamydial infection. If this approximation is correct and 5–10% of all women are infected with *C. trachomatis* once in their lives, the risk of tubal occlusion associated with this sexually transmitted disease would amount to 10–20%!

Clinical signs of genital chlamydial infection are mild and unspecific in most cases. In women, cervical purulent discharge, recurrent lower abdominal pain, irregular vaginal bleeding, moderately elevated erythrocyte sedimentation rate (e. g. 15–25 mm/h), and slight leucocytosis (e. g. 10,000/μl) with normal C-reactive protein are typical and unspecific signs of urogenital infection with *C. trachomatis*. Isolation of *C. trachomatis* from cervical or urethral swabs or urine is proof of the infection. However, failure of antigen detection from these sites does not exclude a chlamydial infection of the upper genital tract [4].

The diagnosis of genital chlamydial infections by antigen detection is complicated by the fact that the number of infectious particles is often low. The sensitivity of cell culture as the gold standard ranges between 70–80% [5, 6]. Direct fluorescence testing (DFA) has a sensitivity in the same range [5], but specificity is below 100% when the cut-off is set below ten elementary bodies. A sensitivity of 70% and specificity of 98% was found for the DFA in a study population with 4.4% prevalence of infection [7]. Enzyme immunoassays (EIA) have a sensitivity in the range of cell culture [6] or less [8] and exhibit a rate of 10–30% false-positive results [9]. PCR offers excellent specificity, but its sensitivity is apparently dependent on the extraction procedure used. In one publication the sensitivity of culture in urogenital specimens (antigen prevalence 23%) was only 70% compared with a self-developed PCR [10]. The commercially available Amplicor-PCR from first-catch urine was 15% more sensitive than culture from urethral swabs of 365 men with an infection prevalence of 10% [11]. However, the Amplicor-PCR was less sensitive than culture for endocervical samples from 587 women with an infection prevalence of 10% presumably

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Table 1: Prevalence of chlamydial IgG in 100 sera of blood donors tested with the Bivalent (LPS extracted *Chlamydia trachomatis L2* elementary bodies), the MIF (highest IgG titre of pool B, C and I), and the ImmunoComb (*Chlamydia trachomatis L2* elementary bodies with LPS).

IgG titre	Bivalent	MIF	ImmunoComb
Negative	90 (90%)	89 (89%)	83 (83%)
1 : 8	3 (3%)	4 (4%)	–
1 : 16	2 (2%)	2 (2%)	–
1 : 32	3 (3%)	1 (1%)	8 (8%)
1 : 64	1 (1%)	4 (4%)	3 (3%)
1 : 128	–	–	4 (4%)
1 : 256	–	–	2 (2%)
1 : 512	1 (1%)	–	–

due to inhibition of the PCR by components of the endocervical mucus [12].

The number of elementary bodies found in the urogenital tract exhibits a considerable natural fluctuation so that around 20% of the originally positive patients turn negative when repeat specimens are taken at control visits a few days later [6, own unpublished observations]. This further complicates the detection of *C. trachomatis* antigen in the urogenital tract.

Since species specific *C. trachomatis* serology is very sensitive and antibodies persist for years, such serologic tests are – at least in principle – very well suited as a supplemental diagnostic tool to rule out or confirm suspicion of an infection with *C. trachomatis*. However, the introduction of species specific serology has been hampered by two facts: the microimmunofluorescence test [13, 14] is not suited for routine laboratories, and the existing routine serology tests employ group-specific lipopolysaccharide (LPS) or even reticulate bodies (contain group specific antigens) as antigen and thus show more or less cross-reactivity with *C. pneumoniae* [15]. Since the prevalence of IgG against *C. pneumoniae* amounts to more than 50% in the healthy population [16, 17] any cross-reactivity with this chlamydial species will lead to unacceptably high ratios of false positives in patient groups with low prevalence of *C. trachomatis*. The species specificity of the Bivalent routine test is evaluated below.

Materials and Methods

The ImmunoComb® Chlamydia Bivalent IgG/IgA (Orgenics, Israel) consists of a white flat plastic comb with 12 teeth. Constant aliquots of *C. trachomatis* L2 elementary bodies (Washington Research Foundation, Seattle) with quantitatively extracted lipopolysaccharide (LPS) are fixed near the tip of each tooth (dots of 3 mm diameter). A second antigenic dot consists of LPS extracted *C. pneumoniae* elementary bodies. A third dot with goat-anti-human IgG serves as a control that the tooth did react with human serum. A prefabricated, disposable reaction chamber with rows A–F and 12 wells in each row is used during the test procedure. After perforation of the covering aluminium sheet with a pipette, 1:32 diluted human serum is filled into wells 3–12 of row A. Positive and negative control sera are added to wells 1 and 2. After incubation at room temperature, the comb is moved from row A to row B for 2 min washing. The comb reacts with alkaline phosphatase conjugated anti-human goat serum in row C. Two washing steps follow in row D and E, 2 min each. Staining is done at room temperature in row F with nitro-blue-tetrazolium (NTB) and 5-bromo-4-chloro-3-indolyl-phosphate (BCIP). The reaction is stopped by moving the comb back to row E. The test is completed within 2h for IgG and 2 1/2 h for IgA. The *C. trachomatis* L2 antigen dot stays white or turns light or dark blue in proportion to the *C. trachomatis* species specific antibody concentration in the human serum. Similarly the *C. pneumoniae* dot reacts to *C. pneumoniae* species specific antibodies in the patient serum. One of the dots may become heavily coloured while the other stays white, which argues in favour of the claimed species specificity of the Bivalent test.

The test result is read by a simple optical reading instrument supplied by the manufacturer. The dot intensities may also be read semi-quantitatively by eye. After reading and marking of the teeth with the patient's serum number, the combs can be stored indefinitely for documentation without fading of the dot colour.

The Bivalent test was interpreted as follows:

Chlamydia trachomatis IgG	Relative absorption	*Chlamydia trachomatis* IgA	Relative absorption
1:8	700–779	1:8	300–449
1:16	780–880	1:16	450–599
1:32	881–1,160	1:32	600–800
1:64	1,161–1,410	≥ 1:64	> 800
1:128	1,411–1,600		
1:256	1,601–1,800		
≥ 1:512	≥ 1,801		

The microimmunofluorescence test (MIF) was performed according to *Wang* and *Graystone* [13]. *C. trachomatis* and *C. pneumoniae* elementary bodies from the Washington Research Foundation in Seattle were used as antigen. The *C. trachomatis* antigen consisted of three different pools: pool B with serovars B, E, D; pool C with serovars C, H, I, J; pool I with serovars G, F, K. Preparation and reading of the MIF was carried out by experienced personnel. The sera were screened at a dilution of 1:8. Positive sera were titrated in serial twofold dilutions up to 1:1,024. Sera were tested for IgG, IgA, and IgM. To exclude false-positive results due to rheumatoid factor, sera with IgM titers of ≥ 1:16 were retested after preabsorption with rheumatoid factor absorbent (Behring, Germany). The highest IgG (IgA) titre of the three *C. trachomatis* pools was chosen for comparison with the Bivalent IgG/IgA.

The ImmunoComb® *C. trachomatis* IgG/IgA (Orgenics, Israel) employs *C. trachomatis* L2 elementary bodies with LPS and thus exhibits some cross-reactivity with *C. pneumoniae* though less than "whole inclusion" tests, e. g. Ipazyme [15]. The results of the ImmunoComb® *C. trachomatis* IgG/IgA ("ImmunoComb") are compared with the ImmunoComb® Chlamydia Bivalent IgG/IgA ("Bivalent") in Tables 1, 5 and 7 to demonstrate the effect of LPS extraction.

Five different patient groups were tested:

1. One hundred blood donors (34 women, 66 men, mean age 30 years)

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2. One hundred and three women with *C. trachomatis* positive cervical smears. *C. trachomatis* antigen was detected by culture and/or direct immunofluorescence (Micro Trak) by a very experienced medical assistant.
3. Fifty-one women with primary or secondary sterility. Pelviscopically both fallopian tubes were completely occluded with no penetration of blue dye from the cavum uteri through the tubes.
4. Fifty-four women with primary or secondary sterility. Pelviscopically both fallopian tubes were open. Blue dye penetrated freely from the cavum uteri through the tubes.
5. One hundred and eleven obstetric patients. Serum samples and cervical smears were taken 6 days post partum. Antigen detection was performed with an ELISA (IDEIA, Boots-Celltech, Slough, UK) and confirmed on a control visit by immunofluorescence (MicroTrak, Syva) or PCR (Amplicor, Roche).

Results

Group 1:100 Blood Donors

In this control group the prevalence of positive IgG (≥ 1:8) was almost identical for the – LPS extracted – ImmunoComb® Chlamydia Bivalent (10%) and the MIF (11%). The ImmunoComb *C. trachomatis* (LPS not extracted) exhibited positive IgG (≥ 1:32) in 17% (Table 1). Thus, about 40% of the positive IgG titers in this test appear to represent LPS cross-reactions with *C. pneumoniae.*

Group 2: 103 Women with Chlamydia trachomatis Positive Cervical Smears

As seen in Tables 2a and b, Bivalent and MIF did not differ significantly for *C. trachomatis* specific IgG. Positive titers (IgG ≥ 1:8) were found in 88% (GMT 1:132) and 85% (GMT 1:76), respectively. However, the prevalence of *C. trachomatis* specific IgA (≥ 1:8) was high for the Bivalent (78%, GMT 1:17) and relatively low for the MIF (24%, GMT 1:7). Positive MIF IgM (≥ 1:8) was seen in 44 patients (43%). MIF IgM was positive (titre 1:8) in five cases with negative IgG and showed high titers of 1:64 in several cases with low IgG (Table 2b).

Group 3: 51 Sterile Women with Both Fallopian Tubes Occluded

As for patients with positive cervical smears, the prevalence of positive IgG (≥ 1:8) correlated well for Bivalent (86%, GMT 1:129) and MIF (84%, GMT 1:65) as shown in Tables 3a and b. Again, positive IgA (≥ 1:8) was significantly more prevalent in the Bivalent (71%, GMT 1:14) than in the MIF (20%, GMT 1:4). MIF IgM titres ≥ 1:8 were seen in 26 patients (51%), in four cases with negative IgG.

Table 2: One hundred and three sera of female patients with *Chlamydia trachomatis* positive cervical smears. Bivalent IgG and IgA are demonstrated in Table 2a, MIF IgG and IgA in Table 2b. MIF IgM titres are shown in parentheses for every MIF IgG titre.

Table 2a

IgG Bivalent IgA	Negative	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	Total
Negative	12	4		6	1				23 (22%)
1 : 8		7	3	5	7	4	3	5	34 (33%)
1 : 16		1	2	5	7	2	1	2	20 (19%)
1 : 32			1	3	4	3	1	4	16 (16%)
1 : 64				1	1	1	1	6	10 (10%)
Total	12 (12%)	12 (12%)	6 (6%)	20 (19%)	20 (19%)	10 (10%)	6 (6%)	17 (17%)	103 (100%)

Table 2b

IgG MIF IgA (IgM)	Negative	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	Total
Negative	15 (10)	10 (4)	10 (6)	17 (8)	15 (15)	6 (10)	4 (4)	1 (2)	78 (76%)
1 : 8	– (5)	– (3)	1 (3)	1 (6)	2 (3)	4 (4)	– (3)		8 (8%)
1 : 16			1	– (2)	1	2 (1)	1		5 (5%)
1 : 32		– (2)	– (2)		1 (3)	3 (1)	2		6 (6%)
1 : 64		– (1)	– (1)	– (2)	2	1	1 (1)	2 (1)	6 (6%)
Total	15 (15%)	10 (10%)	12 (12%)	18 (17%)	21 (20%)	16 (16%)	8 (8%)	3 (3%)	103 (100%)

Group 4: 54 Sterile Women with Both Fallopian Tubes Open

The results are listed in Tables 4a and b. The prevalence of *C. trachomatis* specific IgG (≥ 1:8) is low in this group of sterile women compared with group 3. Positive IgG was measured at almost equal rates in both Bivalent (26%, GMT 1:9) and MIF (22%, GMT 1:9). Prevalence of positive IgA was higher in the Bivalent (13%) than in the MIF (6%), but only by a factor of 2 (13% vs. 6%) instead of 3 (71% vs. 20%), as in groups 2 and 3. MIF IgM was positive in 15 patients (28%) and had a higher prevalence than MIF IgG in this group! Thus, MIF IgM appeared to be unspecific. Thirteen patients with negative MIF IgG (and IgA) showed positive MIF IgM, in four cases at IgM titres of 1:32 (after preabsorption with rheumatoid factor absorbent).

Evaluation of Group 3 and 4 in the Chi-square Test

The statistical evaluation of Bivalent, MIF, and ImmunoComb [15] for the prediction of the tubal status – open or occluded – is demonstrated in Table 5. Positive IgG in the Bivalent, MIF, or ImmunoComb were all highly correlated with tubal occlusion (chi-square 37.0–45.3). The chi-square value of 36.0 for the Bivalent IgA was in the same range whereas the ImmunoComb IgA was less correlated (chi-square 14.7) with the tube status. Surprisingly, positive MIF IgA and IgM showed no significant correlation with tubal occlusion (chi-square less than 6.0, $p > 0.01$). Both ImmunoComb and MIF use elementary bodies containing LPS as test antigen.

Group 5: 111 Obstetric Patients 6 Days post partum

One hundred and eleven obstetric patients were tested with the Bivalent (Table 6a). Six of 111 had *C. trachomatis* positive cervical smears (bold numbers in Table 6a) and were all Bivalent IgG positive. Four of these six were also Bivalent IgA positive. Since one of the patients with positive cervical smears had a Bivalent IgG of only 1:8 with an IgA of 1:16 (MIF IgG and IgA negative), it appears that patients with low Bivalent IgG should be tested for IgA. Fifty obstetric patients were tested both with the Bivalent and the MIF (Table 6b and c). None of the three patients with positive cervical smears had MIF IgA (bold numbers in Table 6c), two had MIF IgM and one was negative in the MIF.

Evaluation of Group 5 in the Chi-square Test

The Bivalent IgG/IgA showed the highest correlation with

Table 3: Fifty-one sera of sterile women with pelviscopically bilaterally *occluded tubes*. Bivalent IgG und IgA are demonstrated in Table 3a, MIF IgG and IgA in Table 3b with MIF IgM in parentheses.

Table 3a

IgG Bivalent IgA	Negative	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	Total
Negative	7		1	3	1	2	1		15 (29%)
1 : 8		1		5	2	1	2	2	13 (25%)
1 : 16		1		1	5	2	5	1	15 (29%)
1 : 32						3		1	4 (8%)
1 : 64			1	1	1			1	4 (8%)
Total	7 (14%)	2 (4%)	2 (4%)	10 (10%)	9 (18%)	8 (16%)	8 (16%)	5 (10%)	51 (100%)

Table 3b

IgG MIF IgA (IgM)	Negative	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	Total
Negative	8 (4)	2 (1)	7 (6)	7 (4)	8 (6)	7 (2)	2 (2)		41 (80%)
1 : 8	– (4)	– (1)	1 (2)	1 (4)	1 (4)	1 (3)	1 (2)		5 (10%)
1 : 16					1				1 (2%)
1 : 32			1		– (1)	1 (2)	1		3 (6%)
1 : 64			– (1)		1	– (2)			1 (2%)
Total	8 (16%)	2 (4%)	9 (18%)	8 (16%)	11 (22%)	9 (18%)	4 (8%)		51 (100%)

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antigen positivity in cervical smears (Table 7a and b). In this low prevalence group stringent specificity for *C. trachomatis* is imperative for a high positive prediction. Here, the disadvantage of the ImmunoComb – LPS containing *C. trachomatis* L2 elementary bodies used as antigen – appears clearly in comparison to the LPS extracted Bivalent test: chi-square 17.1 versus 28.9 for IgG and 23.4 versus 29.2 for IgA (Table 7a). The Bivalent IgG should be considered positive for titres ≥ 1:8, i. e. even for low titres. The Bivalent IgA should also be considered positive for titres ≥ 1:8. In this low prevalence group, Bivalent IgA was less sensitive than IgG (67% vs. 100%), but IgA had the highest positive predictive value for antigen detection in cervical smears (44% vs. 30%).

The MIF IgG showed a clearly higher correlation with cervical smears if only titres ≥ 1:16 were considered positive (Table 7b), and MIF IgA and IgM exhibited no significant correlation with antigen detection (Table 7b). These disadvantages of the MIF may be caused by the genus specific LPS – rather than the species specific MOMP – contained in the elementary bodies used as MIF antigen. Both antigens can only be differentiated by their fluorescence patterns: indistinct, irregular, and less bright for the LPS; sharp, homogeneous, and bright for the MOMP.

Discussion

Serology – even species specific – cannot substitute *C. trachomatis* antigen detection at urogenital sites [18] since positive antigen detection is the only definite proof of active chlamydial infection. But failure of antigen detection does not exclude a genital infection with *C. trachomatis* since successful antigen detection depends on the test quality and the biological activity of the infection. A single routine DFA or EIA detects less than 60% of the actual, active genital chlamydial infections (own unplublished observations). Repeat testing is often not possible and too expensive. Laboratory parameters alone, e. g. blood sedimentation rate or leucocytes in vaginal smears or urine, are too unspecific to justify an extensive search for *C. trachomatis* antigen. Clinical signs – present or absent – are unreliable and unspecific and, at least, exclusion of *C. trachomatis* as the causative agent is very helpful. This everyday clinical dilemma is resolved to a great extent by *C. trachomatis* specific serology.

Lack of antibodies excludes a genital chlamydial infection with a probability of about 99% or more if the prevalence rate of *C. trachomatis* antigen is 10% or less since 90% of the antigen-positive patients exhibit species specific IgG

Table 4: Fifty-four sera of sterile women with pelviscopically bilaterally *open tubes*. Bivalent IgG und IgA are demonstrated in Table 4a, MIF IgG and IgA in Table 4b with MIF IgM in parentheses.

Table 4a

IgG / Bivalent IgA	Negative	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	Total
Negative	40	1	1	4	1				47 (87%)
1 : 8			1	3		1			5 (9%)
1 : 16		1							1 (2%)
1 : 32				1					1 (2%)
1 : 64									
Total	40 (74%)	2 (4%)	2 (4%)	8 (15%)	1 (2%)	1 (2%)			54 (100%)

Table 4b

IgG / MIF IgA (IgM)	Negative	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	Total
Negative	42 (29)	3 (4)	2 (1)	1 (2)	1 (2)	2 (1)			51 (94%)
1 : 8	– (9)	1	– (1)	1		– (1)			2 (4%)
1 : 16									
1 : 32	– (4)								
1 : 64					1				1 (2%)
Total	42 (78%)	4 (7%)	2 (4%)	2 (4%)	2 (4%)	2 (4%)			54 (100%)

Table 5: Prediction of tube status by chlamydial serum antibodies (ab) with three different serological tests. The ImmunoComb Chlamydia Bivalent ("Bivalent") employs LPS extracted *Chlamydia trachomatis* L2 elementary bodies (eb's). The Immunocomb *Chlamydia trachomatis* ("ImmunoComb") uses *Chlamydia trachomatis* L2 elementary bodies containing the genus specific LPS antigen. Chi-square > 10.8 corresponds to $p < 0.001$.

51 occluded tubes versus 54 open tubes	Bivalent – LPS extra eb's – IgG>0	IgG>8	IgA>8	MIF IgG>0	IgG>8	IgA>0	IgM>0	ImmunoComb – eb's with LPS – IgG>0	IgA>0
Occluded / ab pos.	44	42	36	43	41	10	26	45	24
Open / ab pos.	14	12	7	12	8	3	15	16	7
Occluded / ab neg.	7	9	15	8	10	41	25	6	27
Open / ab neg.	40	42	47	42	46	51	39	38	47
Chi-square	**38.6**	**38.0**	**36.0**	**40.5**	**45.3**	**4.77**	**5.93**	**37.0**	**14.7**
Sensitivity	86%	82%	71%	84%	80%	20%	51%	88%	47%
Specificity	74%	78%	87%	78%	85%	94%	72%	70%	87%
Pos. prediction	76%	78%	84%	78%	84%	77%	63%	74%	77%
Neg. prediction	85%	82%	76%	84%	82%	55%	61%	86%	63%

(Tables 2 and 7). According to our results, only very few patients show no or delayed IgG response when *C. trachomatis* is found in urogenital smears. In our experience, most patients have been *C. trachomatis* carriers for months or years before the diagnosis is made, anyway.

On the other hand, positive *C. trachomatis* specific serology supports a positive EIA result to be a true positive, encourages repeat antigen testing (e. g. PCR or LCR) if clinical and/or laboratory signs are present, and calls for a thorough evaluation of the patient's history that often uncovers past episodes of pelvic inflammatory disease. When patients present with sequelae such as tubal occlusion or ectopic pregnancy, chlamydial antibodies are the only diagnostic method to detect the causative agent since cervical smears are negative in almost all of these cases [19].

The ImmunoComb® Chlamydia Bivalent IgG – LPS extracted – correlated very well with the MIF IgG in all high and low prevalence groups evaluated in this study and appeared to be as species specific as the MIF. This was also true for *C. pneumoniae* specific IgG. Patients with *C. trachomatis* positive cervical smears (group 2) showed *C. pneumoniae* specific IgG ≥ 1:16 in 69% in both MIF and Bivalent. The percentages for patients with open tubes were 64% (MIF) and 63% (Bivalent) and for patients with occluded tubes 81% (MIF) and 76% (Bivalent). The prevalence difference – open versus occluded tubes – in *C. pneumoniae* specific IgG was not significant in either the MIF (chi-square 5.2) or in the Bivalent (chi-square 2.2) which underlines that both tests clearly differentiate IgG against both species.

The concordance of MIF and Bivalent *C. trachomatis* specific IgG for each patient was excellent. Of the 358 sera tested in both tests 12 were MIF IgG negative and Bivalent positive (IgG ≥ 1:8), nine were MIF IgG positive (IgG ≥ 1:8) and Bivalent negative. Of this total of 21 discrepant sera 16 differed only in titres 1:8 versus negative.

The low correlation of MIF IgA with tubal status and cervical smears and its low sensitivity compared with the Bivalent IgA was unexpected. MIF IgM performed even worse and appeared to be unspecific since no other MIF, Bivalent or even Ipazyme antibodies and no *C. trachomatis* antigen were detected in many of the MIF IgM positive cases. Whether the MIF IgA and IgM are more influenced by the group specific LPS than MIF IgG remains speculative. The Bivalent IgA showed no signs of unspecificity and proved to be the best serologic predictor of a positive cervical smear. The ImmunoComb® *C. trachomatis* – L2 elementary bodies with LPS – performed well in the high prevalence group of sterility patients (Table 5). This is not surprising since high specificity becomes less important with increasing prevalence. Even sterile women with open tubes had a clearly higher prevalence of *C. trachomatis* antibodies (26%) than obstetric patients (18%) or blood donors (10%). Tubes may be in some cases functionally impaired by a previous chlamydial infection – leading to tubal factor sterility – without being impenetrable for blue dye which is a rather crude method for the determination of tube function. However, in low prevalence groups excellent specificity is mandatory for any high quality diagnostic test. The Bivalent IgG and the MIF IgG were highly concordant in the low prevalence groups, whereas about 30–40% of the ImmunoComb (with LPS) IgG appeared to be false positives in blood donors (Table 1) and obstetric women (Table 7a). Whole inclusion tests, e. g. Ipazyme [15], present with even higher rates of false positives in low prevalence groups which leads to much confusion in clinical practice. Consequently, tests with more or less cross-reactivity with *C. pneumoniae* should not be used in low prevalence groups.

As seen in Table 2, 12% of all patients with *C. trachomatis* positive cervical smears have rather low Bivalent IgG titres of 1:8 though accompanied by positive Bivalent IgA in most cases. Consequently, even low Bivalent IgG titres

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should be an indication of possible antigen presence especially in connection with positive IgA. Generally, high or low titres should not be interpreted as "ongoing acute" or "past inactive" infection. Positive Bivalent *C. trachomatis* IgG indicates a past or ongoing genital chlamydial infection. Antigen detection differentiates between past and ongoing infection though a substantial part of ongoing infections might be missed, depending on the sensitivity of the antigen detection test. In this respect, DNA amplification tests look promising.

The low prevalence group of obstetric women appears to be ideal for the evaluation of *C. trachomatis* serological and antigen detection tests. The Bivalent IgG/IgA will be evaluated together with a DNA amplification test in obstetric women for futher validation of the data presented here.

Table 6: Cervical smears and serum samples were taken from 111 obstetric patients 6 days post partum. All women were analysed in the Bivalent test (Table 6a). Fifty of the 111 women were tested both with Bivalent (Table 6b) and MIF (Table 6 c). Bold numbers mark patients with positive cervical smears, six of 111 (Table 6a) and three of 50 (Tables 6b and 6c). MIF IgM is given in parentheses.

Table 6a

IgG Bivalent IgA	Negative	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	Total
Negative	91	3	1	2 + 1	1	2		1	102 (87%)
1 : 8		1			**1** + 1		1	**1**	5 (5%)
1 : 16		**1**			1				2 (2%)
1 : 32							1		1 (1%)
1 : 64								**1**	1 (1%)
Total	91 (82%)	5 (5%)	1 (1%)	3 (3%)	4 (4%)	2 (2%)	2 (2%)	3 (3%)	111 (100%)

Table 6b

IgG Bivalent IgA	Negative	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	Total
Negative	41	2			1	1		1	46 (92%)
1 : 8		1			**1**			**1**	3 (6%)
1 : 16		**1**							1 (2%)
1 : 32									
1 : 64									
Total	41 (82%)	4 (8%)			2 (4%)	1 (2%)		2 (4%)	50 (100%)

Table 6c

IgG MIF IgA (IgM)	Negative	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	Total
Negative	41 + **1** (33 + **1**)	2 (2)	**1**	1 (1)	**1** (1)		1 (1)		48 (96%)
1 : 8	– (2)	1	– (1)						1 (2%)
1 : 16	– (1)				– (1)				
1 : 32	– (4)	– (1)							
1 : 64	– (1)				1				1 (2%)
Total	42 (84%)	3 (6%)	1 (2%)	1 (2%)	2 (4%)		1 (2%)		50 (100%)

Table 7: Prediction of *Chlamydia trachomatis* positive cervical smears (ag pos.) by positive antibodies (ab pos.) in three different *Chlamydia trachomatis* serological tests (see Table 5). Only 50 of 111 obstetric patients were tested in the MIF (Table 7b). Table 7a shows the results of all 111 women tested both in the LPS extracted ImmunoComb Chlamydia Bivalent ("Bivalent") and the LPS containing ImmunoComb *Chlamydia trachomatis* ("ImmunoComb") test (Table 7a).

Table 7a

111 obstetric patients / 6 antigen positive	Bivalent – LPS extracted eb's – IgG>0	IgG>8	IgA>0	MIF IgG>0	IgG>8	IgA>0	IgM>0	ImmunoComb – eb's with LPS – IgG>0	IgA>0
Ag pos. / ab pos.	6	5	4					6	4
Ag neg. / ab pos.	14	10	5					24	7
Ag pos. / ab neg.	0	1	2					0	2
Ag neg. / ab neg.	91	95	100					81	98
Chi-square	**28.9**	**26.5**	**29.2**					**17.1**	**23.4**
Sensitivity	100%	83%	67%					100%	67%
Specificity	87%	90%	95%					77%	93%
Pos. prediction	30%	33%	44%					20%	36%
Neg. prediction	100%	99%	98%					100%	98%

Table 7b

50 obstetric patients / 3 antigen positive	Bivalent – LPS extracted eb's – IgG>0	IgG>8	IgA>0	MIF IgG>0	IgG>8	IgA>0	IgM>0	ImmunoComb – eb's with LPS – IgG>0	IgA>0
Ag pos. / ab pos.	3	2	3	2	2	0	2	3	2
Ag neg. / ab pos.	6	3	1	6	3	2	8	7	2
Ag pos. / ab neg.	0	1	0	1	1	3	1	0	1
Ag neg. / ab neg.	41	44	46	41	44	45	39	40	45
Chi-square	**14.5**	**11.4**	**36.7**	**6.1**	**11.4**	**0.13**	**4.34**	**12.8**	**14.9**
Sensitivity	100%	68%	100%	67%	67%	0%	67%	100%	67%
Specificity	87%	94%	98%	87%	94%	96%	83%	85%	96%
Pos. prediction	33%	40%	75%	25%	40%	0%	20%	30%	50%
Neg. prediction	100%	98%	100%	98%	98%	94%	97%	100%	98%

Zusammenfassung: *Chlamydia trachomatis* spezies-spezifische Serologie: ImmunoComb® Chlamydia Bivalent versus Mikroimmunfluoreszenztest (MIF). Der ImmunoComb® Chlamydia Bivalent IgG/IgA (Orgenics, Israel) ist ein neuer serologischer Test, der LPS extrahierte *Chlamydia trachomatis* L2 und LPS extrahierte *Chlamydia pneumoniae* Elementarkörperchen auf zwei getrennten Antigenpunkten verwendet. Die Bivalent *C. trachomatis* spezifischen Testergebnisse wurden mit dem Mikroimmunfluoreszenztest (MIF), dem Goldstandard der spezies-spezifischen Chlamydienserologie, verglichen. In Bezug auf *C. trachomatis* IgG zeigte der Bivalent hohe Übereinstimmung mit dem MIF: Der Anteil positiver Titer (IgG ≥ 1:8) lag bei 10% vs. 11% bei 100 Blutspendern, 18% vs. 16% bei 111 Wöchnerinnen (6% Antigenprävalenz), 26% vs. 22% bei sterilen Frauen mit offenen (n = 54) und 86% vs. 84% mit verschlossenen (n = 51) Tuben und 88% vs. 85% bei 103 Frauen mit *C. trachomatis* positivem Zervixabstrich. Überraschenderweise zeigten Bivalent und MiF sehr unterschiedliche IgA Prävalenzen: bei Wöchnerinnen (8% vs. 4%), sterilen Frauen mit offenen (13% vs. 6%) und verschlossenen (71% vs. 20%) Tuben und Frauen mit positivem Zervixabstrich (78% vs. 24%). Der Bivalent IgA Test zeigte sich sensitiver als der MIF IgA bei gleichzeitig höherer Korrelation zu positivem Zervixabstrich bei Wöchnerinnen (Sensitivität 67% vs. 0%, Spezifität 95% vs. 96%, positive Vorhersage 44% vs. 0%, negative Vorhersage 98% vs. 94%) und zum Tubenverschluß bei sterilen Frauen (Sensitivität 71% vs. 20%, Spezifität 87% vs. 94%, positive Vorhersage 84% vs. 77%, negative Vorhersage 76% vs. 55%). MIF IgM erwies sich als diagnostisch wenig hilfreich. Als Ergänzung zum oft schwierigen *C. trachomatis* Antigennachweis erscheint der einfach durchzuführende Bivalent IgG/IgA als sehr nützlich in der Routinediagnostik genitaler Chlamydieninfektionen.

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