

Comparison of Three Commercially Available Peptide-Based Immunoglobulin G (IgG) and IgA Assays to Microimmunofluorescence Assay for Detection of *Chlamydia trachomatis* Antibodies

Servaas A. Morré,¹ Christian Munk,² Kenneth Persson,³ Susanne Krüger-Kjaer,² Rogier van Dijk,¹ Chris J. L. M. Meijer,¹ and Adriaan J. C. van den Brule^{1*}

Department of Pathology, Section of Molecular Pathology, Vrije Universiteit Medical Center, Amsterdam, The Netherlands¹; Danish Cancer Society, Institute of Cancer Epidemiology, Copenhagen, Denmark²; and Department of Clinical Microbiology, Malmö University Hospital, Malmö, Sweden³

Received 29 May 2001/Returned for modification 23 July 2001/Accepted 18 November 2001

Three commercially available, peptide-based enzyme-linked immunosorbent assay (ELISA) systems (*Chlamydia trachomatis* IgG and IgA EIA [CT-EIA; Labsystems OY, Helsinki, Finland], SeroCT IgG and IgA [SeroCT; Savyon Diagnostics Ltd., Ashdod, Israel], and *Chlamydia trachomatis* IgG and IgA pELISA [CT pELISA; Medac, Wedel, Germany]) were evaluated for the detection of serum immunoglobulin G (IgG) and IgA antibodies specific for *Chlamydia trachomatis* and compared to the “gold standard” assay, the microimmunofluorescence (MIF) assay. Serological responses were analyzed in 149 women aged 20 to 30 years. Cervical swabs obtained from these women were examined for *C. trachomatis* by PCR, and 43 were found to be positive. The overall seroprevalence rates detected by CT-EIA, SeroCT, CT pELISA, and the MIF assay were 42, 42, 35, and 39%, respectively, for IgG and 7, 7, 3, and 7%, respectively, for IgA. The IgG seroprevalence rates for the PCR-positive women were two to three times higher than those for the PCR-negative women, i.e., 72 versus 29%, 72 versus 29%, 47 versus 26%, and 74 versus 25% for CT-EIA, SeroCT, CT pELISA, and the MIF assay, respectively. After discrepancy analysis, the sensitivity, specificity, positive predictive value, and negative predictive value were calculated for the IgG assays; for CT-EIA they were 84.7, 98.6, 98.4, and 86.7%, respectively; for CT pELISA they were 71.4, 97.3, 96.2, and 78.3%, respectively; for SeroCT they were 84.7, 98.6, 98.4, and 86.3%, respectively; and for the MIF assay they were 79.2, 83.1, 98.3, and 83.1%, respectively. In conclusion, these peptide-based ELISA systems for the serological detection of *C. trachomatis* infection performed as well as the MIF assay. Since these tests are less time-consuming, less expensive, and easier to perform than the MIF assay, they might be useful in the serodiagnosis of chlamydial infection.

Chlamydia trachomatis infection is the most prevalent sexually transmitted disease in Europe and the United States. In women, this infection can lead to severe sequelae like ectopic pregnancy and tubal infertility. *C. trachomatis* serology has been used for both diagnostic purposes and large epidemiological studies. However, widespread introduction of *C. trachomatis*-specific serology has not gained wide acceptance. The microimmunofluorescence (MIF) assay (10) is still regarded as the “gold standard” in the serological diagnosis of *C. trachomatis* infections. However, the MIF assay is not suited for use by routine laboratories since reading of the specific fluorescence requires a high degree of expertise. Also, current serological tests employ either group-specific lipopolysaccharide (LPS) or reticulate bodies as antigen and thus show cross-reactivity with *C. pneumoniae* (4). Serological cross-reactivity leads to high rates of false-positive results, especially in a population with a low prevalence of *C. trachomatis* infection, since the rates of *C. pneumoniae* seroprevalence are up to 60%. In addition, there have been reports on serological cross-reactivity

due to proteins from other bacteria, e.g., *Acinetobacter* (2).

Several user-friendly enzyme immunoassays with increased specificity have been developed by using LPS-stripped *C. trachomatis* particles (8). Recently, three commercially available assays have been developed by using specific synthetic peptides based on the major outer membrane of *C. trachomatis*. These assays have the potential to be both specific and sensitive. Proper comparison of these serological assays to the MIF assay is strongly needed since these new tests are well standardized, less expensive, and less laborious than MIF.

Therefore, we analyzed these new commercially available, peptide-based enzyme-linked immunosorbent assay (ELISA) systems for the detection of specific serum immunoglobulin G (IgG) and IgA antibodies to *C. trachomatis* in relation to the detection of *C. trachomatis* infections in the corresponding cervical scrapings by PCR. The performances of these new assays were compared to that of the MIF assay.

MATERIALS AND METHODS

Patient population. Sera from 149 women were analyzed for IgG and IgA antibodies against *C. trachomatis*. These 149 women, who were part of a population-based cohort (6), were screened for asymptomatic *C. trachomatis* infections by PCR (performed as described previously [5, 7]). Cervical scrapings were

* Corresponding author. Mailing address: Department of Pathology, Section of Molecular Pathology, University Hospital Vrije Universiteit, De Boelelaan 1117, 1081 HV, Amsterdam, The Netherlands. Phone: 31-20-4440503. Fax: 31-20-4442964. E-mail: vandenbrule@vumc.nl.

TABLE 1. Performances of the three peptide-based serological assays and the MIF assay

Antibody and subject	No. of women with the indicated result by the following assay:											
	CT-EIA (Labsystems)			SeroCT (Savyon)			CT pELISA (Medac)			MIF assay		
	Positive	Grey zone	Negative	Positive	Grey zone	Negative	Positive	Grey zone	Negative	Positive	Unspecific	Negative
IgG												
PCR-positive women (<i>n</i> = 43)	31	1	11	31	1	11	24	4	15	32	2	9
PCR-negative women (<i>n</i> = 106)	31	3	72	31	6	69	28	1	77	26	0	80
Total	62	4	83	62	7	80	52	5	92	58	2	89
IgA												
PCR positive women (<i>n</i> = 43)	5	3	35	3	5	35	1	2	40	5	0	38
PCR negative women (<i>n</i> = 106)	5	3	98	7	2	97	4	0	102	6	0	100
Total	10	6	133	10	7	132	5	2	142	11	0	138

PCR positive for *C. trachomatis* for 43 women and PCR negative for *C. trachomatis* for 106 women.

Serological assays. The following three peptide-based serological assays were compared: Chlamydia trachomatis IgG and IgA EIA (CT-EIA; new version; Labsystems OY, Helsinki, Finland), SeroCT IgG and IgA (SeroCT; Savyon Diagnostics Ltd., Ashdod, Israel), and the Chlamydia trachomatis IgG and IgA pELISA (CT pELISA; Medac, Wedel, Germany). All three tests were performed according to the manufacturers' instructions. An in-house MIF assay was performed as described previously (9). Slides with antigens of *C. trachomatis*, *C. psittaci*, and *C. pneumoniae* (Labsystems OY) were used for this assay. A titer of ≥ 16 was considered diagnostically significant. In addition, the in-house MIF assay and a second MIF assay (the MRL-MIF assay; MRL Diagnostics, Santa Barbara, Calif.) were used for discrepancy analysis. This test was performed with all samples for which there was no concordance with the peptide-based assays.

Statistical analysis. For comparison of the three peptide-based tests to the MIF assay, the sensitivities, specificities, positive predictive values (PPVs), and negative predictive values (NPVs) were calculated by using two-by-two tables. Values in the grey zone (optical densities between the values for negativity and positivity), as defined by the manufacturer, were excluded from these calculations.

Discrepancy analysis was performed by the in-house MIF assay a second time and by the MRL-MIF assay. MIF assay results were considered true-positive results if the second MIF assay or the MRL-MIF assay could confirm the initially positive MIF assay result. After discrepancy analysis, true-positive results were defined as either positivity or negativity by the MIF assay but positivity by at least two of the three peptide-based assays.

To investigate if the *C. trachomatis* serological titers determined by the MIF assay were higher for the women who were PCR positive for *C. trachomatis* than for the women who were PCR negative for *C. trachomatis*, a *t* test for independent samples was performed.

RESULTS

Seroprevalence rates and concordance. The MIF assay with slides with antigens of *C. trachomatis*, *C. psittaci*, and *C. pneumoniae* (Labsystems OY) showed no cross-reactivity with *C. pneumoniae* (data not shown). The IgG and IgA seroprevalence rates for the three peptide-based assays and the MIF assay are shown in Table 1. For all four assays the overall IgG seroprevalence rate was about 40% and the overall IgA seroprevalence rate was about 7%. The IgG seroprevalence rates for the women who were PCR positive for *C. trachomatis* were two to three times higher than those for the PCR-negative women. The IgG seroprevalence rate for the CT pELISA for the PCR-positive women (56% [24 of 43 women]) was lower than that for the other assays (about 72% [31 of 43 women]). The overall IgA seroprevalence rate for the CT pELISA (3%) was also lower than that for the other assays (7%). Women positive for IgA were almost all positive for IgG too: for CT-EIA, 90% (9 of 10); for CT pELISA, 80% (4 of 5); for SeroCT,

100% (10 of 10); and for the MIF assay, 91% (10 of 11). The concordances of the IgG results for all of the different assays are shown in Table 2. Most samples (*n* = 36) were positive by all four tests, whereas 13 were positive by all three peptide-based assays, and 10 samples were positive only by the MIF assay.

Test performances. The sensitivities, specificities, PPVs, and NPVs of the three peptide-based assays and the MIF assay, as calculated after discrepancy analysis, are shown in Table 3. Results are calculated only for the IgG assays since the seroprevalence of IgA was too low to make accurate calculations. Although the results for the four assays were comparable, all four variables analyzed were slightly lower for the CT pELISA. *C. pneumoniae* titers were not responsible for the discrepancies between the results of the tests.

MIF assay titers. The serum *C. trachomatis* IgG titers in the sera of the PCR-positive and -negative women determined by the MIF assay were compared to investigate whether the *C. trachomatis* titers were associated with PCR positivity. Al-

TABLE 2. Concordance of IgG results for all of the different tests for the test population

No. of tests positive	Test with positive result	No. of positive women
4	CT-EIA, CT pELISA, SeroCT, MIF assay	36
3	CT-EIA, CT pELISA, SeroCT	13
	CT-EIA, CT pELISA, MIF assay	1
	CT-EIA, SeroCT, MIF assay	7
2	CT-EIA, SeroCT	2
	CT-EIA, MIF assay	2
	SeroCT, MIF assay	2
1	CT-EIA	1
	CT-pELISA	2
	SeroCT	2
	MIF	10
0	Combinations of results in the grey zone without the other tests being positive	6
0	None positive or no grey zone	65
Total		149

TABLE 3. Sensitivities, specificities, PPVs, and NPVs of the three IgG peptide-based assays and MIF assay after discrepancy analysis

Test	No. of samples with the following result ^a :					Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	True positive	False positive	True negative	False negative	Grey zone ^b				
CT-EIA	61	1	72	11	4	84.7	98.6	98.4	86.7
SeroCT	61	1	69	11	7	84.7	98.6	98.4	86.3
CT pELISA	50	2	72	20	5	71.4	97.3	96.2	78.3
MIF	57	1	74	15	2	79.2	83.1	98.3	83.1

^a A total of 149 samples were tested by each assay.

^b Values in the grey zone for each test are excluded from these calculations.

though the mean titers in the sera of the PCR-positive women were found to be slightly higher than those in the sera of the PCR-negative women, the difference was not statistically significant by the *t* test for independent samples ($P = 0.135$).

DISCUSSION

This is the first study to compare three recently introduced commercially available peptide-based serology assays to the MIF assay for the detection of antibodies to *C. trachomatis*. In general, these peptide-based assays performed as well as the MIF assay. Since these tests are easier to perform than the MIF assay, they might be good alternatives to the MIF assay for the detection of *C. trachomatis* antibodies.

In general, the seroprevalence rates and performances of the recently introduced peptide-based assays for the assay of IgG were comparable to those of the MIF assay. Slightly lower *C. trachomatis* prevalence rates and sensitivities were found by the CT pELISA than by the two other peptide-based assays. The samples were retested blindly (Medac), and IgG prevalence rates (40%) and IgG sensitivities (77%) comparable to those obtained by the two other peptide-based assays obtained in the first analysis were found. Although possible differences between batch numbers, buffers, and controls were analyzed, the reason for the difference in the results that were obtained could not be identified. The IgA prevalence rate was too low (7%) to calculate test performances due to the small number of IgA-positive patients. As for the IgG seroprevalence rate, the IgA seroprevalence rate obtained by the CT pELISA was slightly lower than the rates obtained by the other assays. Retesting of the samples for IgA (Medac) resulted in an IgA seroprevalence (9%) comparable to that determined by the CT pELISA.

Thus far, few studies have compared these new serological assays with the MIF assay. Antilla et al. (1) showed that the CT-EIA was more sensitive than the MIF assay in a study that analyzed the influence of *C. trachomatis* on the risk for the development of cervical cancer. Gijzen et al. (A. P. Gijzen, V. J. Goossens, J. A. Land, J. L. H. Evers, and C. A. Bruggeman, Proc. Fourth Meet. Eur. Meet. Eur. Soc. Chlamydia Res., p. 101, 2000) compared the MIF assay to the SeroCT assay and found no significant differences in the sensitivities and specificities between the two assays for patients with tubal factor infertility. Maass et al. (M. Maass, D. Franke, S. Birkelund, G. Christiansen, K. Persson, and M. Böttcher, Proc. Fourth Meet. Eur. Meet. Eur. Soc. Chlamydia Res., p. 112, 2000) compared the MIF assay to the CT pELISA and showed that the assays had good reproducibilities and good overall precisions and

concluded that, due to the synthetic antigen, the assay showed excellent specificity. Finally, Persson and Boman (9) compared the MIF assay to the CT pELISA for patients with tubal factor infertility and controls and showed that the results of the CT pELISA correlated well with the antibody results for *C. trachomatis* obtained by the MIF assay but did not correlate at all with the antibody results for *C. pneumoniae* obtained by the MIF assay.

In the present study, the women were also tested by PCR for the presence of *C. trachomatis* in the corresponding cervical scrapings. The seroprevalence rates for the IgG assays were more than twice as high for women with PCR-positive cervical scrapings than for women with PCR-negative cervical scrapings. The seroprevalence rates for women confirmed to be positive for *C. trachomatis* by PCR in our study (IgG seroprevalence rate range, 72 to 74%; IgA seroprevalence rate range, 7 to 12%) were comparable to those in the literature for women confirmed to be positive for *C. trachomatis* (by culture and direct immunofluorescence [Micro Trak]): IgG seroprevalence rate by the MIF assay, 85%; IgA seroprevalence rate by the MIF assay, 4 to 24% (3). However, not all PCR-positive samples were IgG positive, suggesting a first infection with *C. trachomatis*, an infection with *C. trachomatis* at another site (e.g., an urethral or ocular infection), or a very low IgG response (below the detection level).

The three commercially available assays performed equally well when large numbers of samples were tested and offer the possibility for automation, which is important for large epidemiological studies. Furthermore, in contrast to the MIF assay, objective reading of the results is possible. The prevalence of positive serological results for patients with *C. trachomatis*-positive cervical scrapings (as determined by PCR) is significantly higher (72%) than that for women negative by PCR (28%), and the results obtained by these new assays were comparable to those of the MIF assay, supporting the validities of these new assays. Data were obtained in the present study by using sera from asymptotically infected women; future studies should address both symptomatically infected women and women with late complications to further compare the performances of these new peptide-based assays to the performance of the MIF assay. The peptide-based serological assays might be valuable for epidemiological studies for the analysis of past and current *C. trachomatis* infections.

In conclusion, the three new peptide-based assays performed equally as well as or even slightly better than the MIF assay for the detection of antibodies to *C. trachomatis*. Since these tests are well standardized, less expensive, and less labo-

rious than the MIF assay, they might be good alternatives to the MIF assay for the detection of *C. trachomatis* antibodies.

ACKNOWLEDGMENTS

This work was partly supported by ZON (Prevention Fund) grant 98-1-571, Den Haag, The Netherlands.

We thank LabSystems (CT-EIA), Savyon (SeroCT), and Medac (CT pELISA) for providing the *C. trachomatis* IgG and IgA serology kits and reagents for performance of the comparison.

REFERENCES

1. Antilla, T., P. Saikku, P. Koskela, A. Bloigu, J. Dillner, I. Ikäcimo, E. Jellum, M. Lehtinen, P. Lenner, T. Hakulinen, A. Näävaen, E. Pukkala, S. Thoresen, L. Youngman, and J. Paavonen. 2001. Serotypes of *Chlamydia trachomatis* and risk for development of cervical squamous cell carcinoma. *JAMA* **285**: 47–51.
2. Brade, H., and H. Brunner. 1979. Serological cross-reactivity between *Acinetobacter calcoaceticus* and chlamydia. *J. Clin. Microbiol.* **10**:819–822.
3. Clad, A., H. Freidank, J. Plünnecke, B. Jung, and E. E. Petersen. 1994. *Chlamydia trachomatis* species specific serology: ImmunoComb *Chlamydia* Bivalent versus Microimmunofluorescence (MIF). *Infection* **22**:165–173.
4. Forsey, T. 1987. Antibodies to *Chlamydia trachomatis*. *Genitourin. Med.* **63**:711–716.
5. Jacobs, M. V., A. J. C. van den Brule, P. J. F. Snijders, T. J. M. Helmerhorst, C. J. L. M. Meijer, and J. M. M. Walboomers. 1996. A non-radioactive PCR enzyme-immunoassay enables a rapid identification of HPV 16 and 18 in cervical scrapes after GP5+/6+ PCR. *J. Med. Virol.* **49**:223–229.
6. Kjaer, S. K., A. J. C. van den Brule, J. E. Bock, P. A. Poll, G. Engholm, M. E. Sherman, J. M. M. Walboomers, and C. J. L. M. Meijer. 1996. Human papillomavirus—the most significant risk determinant of cervical intraepithelial neoplasia. *Int. J. Cancer* **65**:601–606.
7. Morré, S. A., P. Sillekens, M. V. Jacobs, P. van Aarle, S. de Blok, B. van Gemen, J. M. M. Walboomers, C. J. L. M. Meijer, and A. J. van den Brule. 1996. RNA amplification by nucleic acid sequence-based amplification with an internal standard enables reliable detection of *Chlamydia trachomatis* in cervical scrapings and urine samples. *J. Clin. Microbiol.* **34**:3108–3114.
8. Ossewaarde, J. M., A. De Vries, J. A. R. van den Hoek, and A. M. van Loon. 1994. Enzyme immunoassay with enhanced specificity for detection of antibodies to *Chlamydia trachomatis*. *J. Clin. Microbiol.* **32**:1419–1426.
9. Persson, K., and J. Boman. 2000. Comparison of five serologic tests for diagnosis of acute infections by *Chlamydia pneumoniae*. *Clin. Diagn. Lab. Immunol.* **7**:739–744.
10. Wang, S.-P., J. T. Grayston, C.-C. Kuo, E. R. Alexander, and K. K. Holmes. 1977. Serodiagnosis of *Chlamydia trachomatis* infection with the microimmunofluorescence test, p. 237–248. *In* D. Hobson and K. K. Holmes (ed.), *Nongonococcal urethritis and related infections*. American Society for Microbiology, Washington, D.C.