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Detection of Endocervical Anti-*Chlamydia trachomatis* Immunoglobulin A in Pregnant Women by a Rapid, 6-Minute Enzyme-Linked Immunosorbent Assay: Comparison with PCR and Chlamydial Antigen Detection Methods

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There is a need for a rapid, uncomplicated, and inexpensive test for *Chlamydia trachomatis* infection in women. We evaluated the ability of a 6-min enzyme-linked immunosorbent assay (ELISA) that requires no laboratory equipment (IgA Rapid SeroTest; Savyon Diagnostics) to detect *C. trachomatis* immunoglobulin A (IgA) in the endocervices of 167 inner-city pregnant women and compared the results with DNA amplification (Amplicor PCR; Roche Diagnostics) and antigen detection (Chlamydiazyme; Abbott Laboratories) performed on the same women. Anti-*C. trachomatis* IgA was detected in the cervices of 32 women (19.2%). Samples from 23 women (13.8%) were PCR positive, while chlamydial antigen was present in 20 women (12.0%). There was only 1 sample (4.3%) that was positive by PCR but negative by ELISA; 10 samples were ELISA positive and PCR negative. In contrast, seven samples (30.4%) were PCR positive but Chlamydiazyme negative and four were Chlamydiazyme positive and PCR negative. Compared to PCR, the IgA ELISA had a sensitivity of 95.7%, a specificity of 93.1%, a positive predictive value of 68.8%, and a negative predictive value of 99.3%. The antigen assay had a sensitivity of only 69.6%, a specificity of 97.2%, a positive predictive value of 80.0%, and a negative predictive value of 95.2%. In high-risk groups where laboratory testing is not available, or where the patient might not return to obtain her testing result and be treated, the Rapid IgA SeroTest is a viable alternative for detection of cervical *C. trachomatis* in pregnant women.

Chlamydia trachomatis, a prevalent sexually transmitted bacterial pathogen, is associated with infertility due to fallopian tube occlusion (4), ectopic pregnancy (4), and adverse pregnancy outcome (9, 11, 16, 17). In the majority of women infected by this organism, symptoms are minimal or nonexistent (4). Therefore, screening of women at risk for chlamydial infection has been advocated (5, 12, 14).

Women at highest risk for a chlamydial genital tract infection often have the least access to health care services and/or are difficult to contact with laboratory results or to schedule a subsequent appointment for antibiotic treatment. There is a need, therefore, for a rapid, simple, and accurate *C. trachomatis* test that can be performed outside of a laboratory setting while the woman is still in the clinical facility.

Currently, *C. trachomatis* detection requires laboratory expertise. The requirement for intracellular growth on cultured mammalian cells makes detection of *C. trachomatis* by culture technically difficult and time consuming. Direct detection of *C. trachomatis* in endocervical samples with an antichlamydial monoclonal antibody or a chlamydial DNA probe is more rapid than culture but has a lower sensitivity (6, 7) and still requires laboratory equipment. Development of DNA amplification assays has further increased the sensitivity of endocervical *C. trachomatis* detection (1, 2). However, expensive supplies and equipment, as well as technically proficient personnel, are required to obtain accurate results.

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The value of anti-*C. trachomatis* endocervical immunoglobulin A (IgA) antibody determination for diagnosis of a current infection remains controversial. McComb et al., using a detection assay that did not differentiate between IgA and IgG antibodies, demonstrated that the presence of antichlamydial immunoglobulins in the cervix, but not in the circulation, correlated with chlamydial isolation from the cervix (8). Brunham et al. also reported that cervical antichlamydial immunoglobulin was more specific than serum antibody in detecting a culture-positive infection (3). In contrast, a third study did not find cervical antibodies useful in the diagnosis of a *C. trachomatis* infection in a low-prevalence population (13).

In the present study, we evaluated the ability of a rapid enzyme-linked immunosorbent assay (ELISA) for cervical antichlamydial IgA to detect PCR-positive *C. trachomatis* endocervical infections in inner-city pregnant women.

MATERIALS AND METHODS

Subjects. Testing was performed on 167 pregnant women seen at the Jersey City Medical Center. The racial composition was 48.8% Hispanic, 37.8% black, 7.3% Caucasian, and 6.1% Asian. Mean ages were 24.3 (Hispanic), 24.0 (black), 29.2 (Caucasian), and 27.4 (Asian) years. Most (>95%) were unmarried and receiving public assistance. Subsequent pregnancy outcome data was not available.

Sample collection. Three samples were collected from each pregnant woman by inserting Dacron swabs into the endocervix and rotating the swabs 360° prior to removal. In random order, one swab was placed in an Amplicor collection tube (Roche Diagnostics, Totowa, N.J.) for PCR analysis, a second swab was placed in Chlamydiazyme storage reagent (Abbott Laboratories, North Chicago, Ill.) and sent to the clinical microbiology laboratory for chlamydial antigen detection, and the third swab was placed in 0.5 ml of phosphate-buffered saline (PBS), the liquid was extracted from the swab with a Pasteur pipette, the sample was microcentrifuged, and the supernatant was frozen at −80°C until tested for antichlamydial IgA.

Chlamydia testing. Samples were tested for *C. trachomatis* DNA by PCR using the commercial Amplicor assay kit (Roche Diagnostics) according to the manufacturer's instructions. Similarly, chlamydial antigen was detected by using the commercial Chlamydiazyme kit (Abbott Laboratories) according to the manufacturer's instructions.

Antichlamydial cervical IgA was detected by the commercially available Chlamydia IgA Rapid SeroTest (Savyon Diagnostics, Ashdod, Israel). Briefly, the kit contains 12 paddles, each composed of 4 wells that are coated with antigens purified from the L2 serovar of *C. trachomatis*. To perform the assay, 50 μl of undiluted cervical sample in PBS was placed in duplicate wells and a cut-off control serum was placed in a third well, while two other wells contained positive- and negative-control sera. Controls were supplied by the manufacturer. IgA antibody-positive and -negative cervical samples that we previously tested using a different assay (17) were also utilized as additional controls. When multiple samples were analyzed, each paddle contained a cut-off control but the positive and negative controls were utilized only once with every assay. After 2 min at room temperature, the liquid was discarded and the wells were washed with the supplied wash buffer. Aliquots (50 μl) of a solution containing horseradish peroxidase (HRP)-conjugated antibody to human IgA were then added for a second 2-min incubation. The wells were again thoroughly washed, and 50 μl of an HRP substrate was added. Following 2 min at room temperature, the liquid was again discarded and a stop solution was added to the wells. The intensity of the blue color change was compared to the colors of the controls. Assays in which the negative control was colorless, the positive control was dark blue, and the cut-off control was light blue were considered valid. A positive result was one in which the visual intensity of the blue color was greater than that of the cut-off control. Duplicate assay results were always consistent.

Samples yielding discordant results for any two *Chlamydia* detection assays were reanalyzed by the same tests and, in addition, were tested by PCR using primer pairs specific for a region of the chlamydial major outer membrane protein (MOMP) gene (15). IgA-positive, PCR-negative samples were also retested by Amplicor PCR after the sample was spiked with *C. trachomatis* elementary bodies.

Statistics. Differences between groups were evaluated by Fisher's exact test.

RESULTS

C. trachomatis DNA was detected by PCR in the endocervices of 23 (13.8%) of the pregnant women tested. Chlamydial antigen was detected in cervices from 20 (12.0%) of the women, while 32 (19.2%) were positive for antichlamydial cervical IgA. There was no relation between race and chlamydial prevalence by any of the assays.

Repeat analyses of discordant results were performed. To rule out the presence of PCR inhibitors in samples that were negative by PCR for chlamydial cryptic plasmid DNA but positive in the antigen or antibody assays, purified *C. trachomatis* was added to aliquots of each sample and this PCR was repeated. In each case, chlamydial DNA was readily detected by the Amplicor assay. Other aliquots were tested by PCR with primer pairs specific for a region of the MOMP gene. Each of the antigen- or antibody-positive samples that were previously PCR negative remained negative for the presence of the MOMP gene. Repeat analyses by each of the assays in PCR-positive but antigen- or antibody-negative samples confirmed the original findings.

Only 1 woman of 23 (4.3%) was positive by PCR but lacked detectable IgA antibodies. This was significantly less ($P = 0.04$) than the seven women (30.4%) who were positive by PCR but negative for detectable chlamydial antigen.

There were 10 women who were positive for antichlamydial IgA but negative by PCR and by antigen assay. Similarly, four women were positive only in the chlamydial antigen detection assay. Relative to PCR, the IgA assay had a sensitivity of 95.7%, a specificity of 93.1%, a positive predictive value of 68.8%, and a negative predictive value of 99.3%. The antigen assay had a sensitivity of 69.6%, a specificity of 97.2%, a positive predictive value of 80.0%, and a negative predictive value of 95.2% (Table 1).

TABLE 1. Comparison of *C. trachomatis* detection by PCR, cervical IgA antibodies, and cervical antigen[a]

Test result	PCR result		Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
	Positive	Negative				
IgA			95.7	93.1	68.8	99.3
Positive	22	10				
Negative	1	134				
Antigen			69.6	97.2	80.0	95.2
Positive	16	4				
Negative	7	140				

[a] Test results are given as numbers of women found positive and negative for *C. trachomatis.*

DISCUSSION

The IgA Rapid SeroTest detected endocervical *C. trachomatis* infections in 22 of 23 (95.7%) pregnant women whose infections were identified by PCR. This was a higher percentage than the number of PCR-positive women who could be identified by a *Chlamydia* antigen-based assay. Thus, in the absence of even the most basic laboratory facilities, *C. trachomatis* could be detected with high sensitivity by this qualitative cervical IgA ELISA. This assay, however, had a positive predictive value of only 68.8%, indicating that some women with no evidence of endocervical chlamydial DNA will, nevertheless, be scored as harboring this organism.

In the 10 women positive for antichlamydial IgA but negative for chlamydial DNA, the possible presence of PCR inhibitors in the samples was ruled out. Alternative explanations for this finding are that (i) there was a recently cleared chlamydial infection in these women and the IgA immune response had not yet subsided, (ii) there was a chlamydial infection above the level of the endocervix that, nevertheless, induced a cervical immune response, or (iii) although none of the cervical samples was visibly contaminated with blood, the possible presence of occult blood contamination in women previously exposed to *C. trachomatis* or to another chlamydial species could lead to a positive cervical antibody test. *Chlamydia* antibody ELISAs are genus specific, not species specific, and so women infected with *C. pneumoniae* or *C. psittaci* and who have antibodies to these organisms circulating may be scored as false positives in *C. trachomatis* antibody testing (10). At present we cannot differentiate between these alternatives. Sera from these women were not available for analysis.

The IgA Rapid SeroTest appeared to offer a convenient and sensitive means to test for *C. trachomatis* in nonlaboratory settings (i) in women in high-prevalence risk groups who may not be available for treatment at a later date if their *C. trachomatis* testing is positive or (ii) in situations where laboratory testing is not available. However, treatment based solely on the antibody findings, although likely to identify those women with PCR-positive infections, may result in unnecessary antibiotic usage in some cases.

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