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Comparison of a Polymer Conjugate-Enhanced Enzyme Immunoassay to Ligase Chain Reaction for Diagnosis of *Chlamydia trachomatis* in Endocervical Swabs

MAX CHERNESKY,[1]* DAN JANG,[1] DEBBY COPES,[2] JAY PATEL,[3] ASTRID PETRICH,[1] KATHLEEN BIERS,[3] ARLENE SPROSTON,[3] AND JULIUS KAPALA[3]

McMaster University and St. Joseph's Hospital, Hamilton,[1] Choice in Health Clinic, Toronto,[2] and Gamma-Dynacare Medical Laboratories, Brampton,[3] Ontario, Canada

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Two endocervical swabs from each of 1,123 women were collected into manufacturer-supplied transport tubes and tested for *Chlamydia trachomatis* by a polymer conjugate-enhanced (PCE) enzyme immunoassay (EIA) (IDEIA PCE Chlamydia; DAKO) and a ligase chain reaction assay (LCx Chlamydia; Abbott). After confirmation by the EIA blocking test, the sensitivity of the IDEIA PCE remained at 91.8% and the specificity increased from 98.2 to 99.8% compared to LCx.

Nucleic acid amplification (NAA) assays for *Chlamydia trachomatis* are more sensitive than culture (2, 15), enzyme immunoassay (EIA) (5, 17), and nucleic acid hybridization (3, 8) and can be used successfully on noninvasive specimens such as urine (1, 11) and vaginal (4), vulvar (12), or introital (16) swabs. These qualities, together with high specificity, provide impetus for using NAA tests such as the ligase chain reaction (LCR) to screen asymptomatic populations at risk. The common limiting factors for NAA tests are increased costs for the assays and lower levels of throughput.

The original 96-well-plate version of the EIA from DAKO, called IDEIA, was introduced in the 1980s. Following extensive evaluations compared with culture and NAA assays (6, 9), IDEIA was grouped with other EIAs, after confirmatory blocking and expansion of the "gold standard," as having a sensitivity of 60 to 70% (10). The manufacturer has since reconfigured the assay by incorporating dual amplification of the indicator system. This polymer conjugate enhancement (PCE) system consists of a dextran backbone to which anti-chlamydial lipopolysaccharide monoclonal antibodies and alkaline phosphatase are bound. For every immune complex interaction, multiple molecules of alkaline phosphatase are available to drive the signal generation in an enzyme-amplified color development system. The performance of the IDEIA PCE Chlamydia assay (DAKO) has been reported for detecting chlamydial antigens in female cervical and vaginal swabs (13, 14) and in male first-void urine (14) compared to PCR (AMPLICOR). The purpose of our study was to compare the IDEIA PCE Chlamydia assay and the LCR test (LCx Chlamydia; Abbott) performed on endocervical swabs collected from women attending a birth control clinic.

The study, which received ethics approval from the St. Joseph's Hospital investigational review committee, took place from April to November 1999. Two endocervical swabs were collected into manufacturer-supplied transport tubes from each of 1,123 consenting women of childbearing age attending a suburban clinic. The order of sampling was reversed after enrolling each 500 patients. All of the samples were held at 4°C, received in the laboratory within 24 h, and then processed within 48 h.

The specimens were tested for *C. trachomatis* by LCR (LCx Chlamydia) and by EIA (IDEIA PCE Chlamydia) according to each manufacturer's instructions for cervical swab testing. All positive samples in the IDEIA PCE test were retested with and without IDEIA PCE Chlamydia blocking reagents. A reduction in the EIA signal of at least 40% confirmed a positive EIA result. Samples which had discordant results were reexamined for elementary bodies (EBs) by the Microtrak (Dade Behring) direct fluorescent-antibody reagent, with the presence of two or more EBs considered positive. Discordant samples without EBs were tested for DNA purified from Qiagen-extracted IDEIA PCE swab material in an in-house PCR measuring *C. trachomatis* plasmid DNA (7).

After confirmatory testing and discordance analysis, the prevalence of *C. trachomatis* infection in this population of women was 4.4% (49 of 1,123). Table 1 shows the performance of the IDEIA PCE Chlamydia assay compared to the LCx Chlamydia assay before and after the confirmatory blocking assay was performed. Both IDEIA PCE and LCx identified 45 infected women. LCx also identified 4 extra women, and IDEIA PCE identified an additional 19. With the LCx results set as the reference standard and no blocking confirmatory testing performed, the sensitivity of the EIA was 91.8% and the specificity was 98.2%. The confirmatory blocking test eliminated 17 of the false-positive results, leaving only two extra positives by IDEIA PCE. This adjusted the specificity to 99.8%, while the sensitivity remained at 91.8%. The results of further testing performed on the discordant specimens showed that the four LCx-positive, EIA-negative specimens had either low levels of EBs or a positive PCR test. The two EIA-positive, LCx-negative specimens were without EBs and were negative by PCR.

Tanaka and coworkers (13) showed the IDEIA PCE test to

* Corresponding author. Mailing address: Medical Microbiology Service, St. Joseph's Hospital, 50 Charlton Ave. East, Room L424, Hamilton, Ontario L8N 4A6, Canada. Phone: (905) 521-6021. Fax: (905) 521-6083. E-mail: chernesk@mcmaster.ca.

TABLE 1. Sensitivity and specificity of IDEIA PCE Chlamydia compared to LCx Chlamydia before and after confirmation with the blocking reagent and discordance analysis

IDEIA PCE result	No. of samples with the following LCx result:		% Sensitivity	% Specificity
	Positive	Negative		
Before confirmation				
Positive	45	19	91.8 (45/49)	98.2 (1,055/1,074)
Negative	4	1,055		
After confirmation				
Positive	45	2[b]	91.8 (45/49)	99.8 (1,072/1,074)
Negative	4[a]	1,072		

[a] The four false-negative IDEIA PCE samples were either positive for EBs or PCR positive.
[b] The two false-positive IDEIA PCE samples were negative for EBs and by PCR.

have a sensitivity of 85.7% on cervical swabs from asymptomatic prostitutes with a chlamydia prevalence of 20%, compared to a sensitivity of 91.4% for the AMPLICOR PCR assay. The specificities of the two assays were 99.2 and 100%, respectively. In a second study (14) using cervical swabs with a similar population, this group showed even higher sensitivities of 95% for IDEIA PCE and 100% for AMPLICOR PCR. Other types of specimens were also tested in these two studies: reported sensitivities of the IDEIA PCE test were 88.8% for vaginal swabs and 91.4% for male first-void urine specimens. These values approximate those achieved by NAA assays performed on noninvasive specimens which may have amplification inhibitors. These results are encouraging and warrant further studies on noninvasive specimens. Kit costs vary according to country and laboratory testing volume. Processing of 91 specimens and controls takes about 2 h of technician time, and the assay results are ready in about 5 h. All positive results need to be confirmed, which increases the time appropriately.

An important practical issue which emerged from our study is the need to perform the IDEIA PCE confirmatory test. Without performance of the blocking assay, an additional 17 false positives would have been reported. A specificity of 99.8% is respectable for any assay. The two apparent false positives may have contained sufficient amounts of NAA inhibitors to negate a positive confirmatory result in the discordance analysis. There was too little of the discordant specimens remaining to allow spiking experiments to be performed for the demonstration of inhibitors. Therefore, we were unable to demonstrate that these samples were not true positives.

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