A Comparison of Rapid Enzyme Immunoassay Tests for the Detection of *Chlamydia trachomatis* Cervical Infections

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Two rapid enzyme immunoassay test kits were compared with culture for the detection of Chlamydia trachomatis *endocervical infections. Endocervical samples for* C trachomatis *culture and the two enzyme immunoassay tests were evaluated from 502 county health department and Planned Parenthood patients. The prevalence of infection in this population was 12%. Sensitivity and specificity of the Abbott TestPack Chlamydia were 51.7% and 99.5%, respectively, and of the Kodak Surecell Chlamydia were 76.7% and 98.6%, respectively. The positive and negative predictive values for TestPack were 93.9% and 93.8%, and for Surecell were 88.5% and 96.9%, respectively.*

Additionally, an in vitro investigation was used to evaluate whether typical office staff (physicians, nurses, medical technicians, receptionists, and radiology technicians) were able to perform competently the tests in each kit. Office personnel tested 12 dilutions of a C trachomatis *stock sample or negative control sample as unknowns for each kit in the in vitro investigation. There were no differences among office staff in performance when compared for each test kit.*

Selective use of these enzyme immunoassay tests for high-risk patients in a family practice population that has a high prevalence of patients with C trachomatis *infection may be helpful when rapid test results are required and cultures are not feasible. After appropriate training, most physician office personnel were equally able to perform the enzyme immunoassay tests evaluated.* **J Fam Pract 1990; 31:597-601.**

In the past decade, antigen-based tests for *Chlamydia trachomatis* have demonstrated clinical value by their reliable ability to detect genital infections in women.[1–4] Until recently, antigen detection methods were suitable primarily for high-volume hospital and reference laboratories or where skilled technicians and special equipment were located. Specimens collected from physician offices and small clinics required shipping to large laboratories for testing. Although these enzyme immunoassay and direct fluorescent antibody tests could be processed in less than 4 hours, the actual turnaround time for notification to the practicing physician was usually a minimum of 24 hours. While this delay is an improvement over the 48- to 96-hour wait for *C trachomatis* culture results, there can be obvious inconveniences.

Two *C trachomatis* enzyme immunoassay (EIA) kits, the TestPack Chlamydia (Abbott Laboratories, Chicago, Ill) and Surecell Chlamydia (Eastman Kodak, Rochester, NY), are commercially available. Both offer rapid test processing, with less than 25 minutes to completion. Each self-contained kit is designed for simplicity of use and requires no specialized training. The importance of these kits for physician office and small clinic laboratories, especially rural practice sites, is evident.

The purpose of the investigation reported here was a comparative evaluation of these two enzyme immunoassay test kits for the detection of *C trachomatis* endocervical infections. To date, there are no published comparisons available to clinicians.

In addition, the practice of presumptive treatment of *C trachomatis* cervicitis based on historical and clinical find-

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ings was evaluated. This common clinical practice originated from the previous lack of test availability resulting from remoteness of location, financial constraints, or the absence of a skilled medical technician or specialized equipment.

An in vitro investigation evaluated the test performance of staff members of a typical office-based practice. After receiving the usual training by the test kit sales representative, office staff performed the two enzyme immunoassay tests and were evaluated for reliability and accuracy of results.

METHODS

Patient Population

Women of varied ethnic backgrounds between 18 and 35 years of age were asked to participate in this investigation. Subjects had routine prenatal, family planning, preabortion, or sexually transmitted disease clinic appointments at the Richmond County Health Department or Planned Parenthood of Augusta, Georgia.

Inclusion criteria were that the subject be a woman 18 years of age or older who met two of five previously established criteria for genital *C trachomatis* infection selective screening.[5] These criteria included age less than 25 years old, oral contraceptive use or no contraceptive use, new sexual partner within the previous 2 months, cervical friability, and clinical evidence of cervical mucopus. Exclusion criteria were the use of antibiotics within the previous 4 weeks or recent douching.

Study Design

Clinicians obtained a standardized history, secured informed consent, and performed a pelvic examination. Four swabs were used to obtain endocervical samples from each patient. The first and second swabs were used for *Neisseria gonorrhoeae* and *C trachomatis* cultures, respectively. The third and fourth swab collections were consecutively alternated for the two EIA test kits being evaluated.

Specimens for chlamydial testing were kept refrigerated at 4°C, collected daily, and hand delivered in a cold pack to the respective laboratories. Chlamydial cultures and EIA tests were performed on the same day as the collection.

Cervical characteristics were noted and recorded. Clinicians indicated whether presumptive treatment for *C trachomatis* infection was initiated based on historical and clinical findings.

Before testing clinical specimens by TestPack and Surecell, each medical technician demonstrated proficiency by performing a coded proficiency panel. The panel consisted of 10 positive, weakly positive, or negative unknown samples.

Clinicians and medical technologists were blinded from results. The two EIA tests and the McCoy cell cultures were processed at separate laboratories.

Phase II

Operator performance variability was evaluated by an in vitro investigation. A single-strain *C trachomatis* solution was serially diluted tenfold to obtain the lowest concentration of organism detected by both the TestPack and Surecell EIA kits. This dilution was designated as a weakly positive sample. A half-log, less dilute solution was designated the positive sample. A negative sample was also created. Collection kit swabs were inoculated with one of the in vitro dilutions and then distributed to the participants as unknowns. The operators consisted of personnel commonly found in a medical office practice: 2 medical technologists, 2 physicians, 1 licensed practical nurse, and 1 registered nurse, 1 receptionist, and 1 x-ray technologist. Each individual processed 12 unknown samples for each of the EIA tests.

Laboratory Methods

On receipt in the laboratory, specimens were spun and inoculated into duplicate 1-dram vials containing coverslips of normal-density McCoy cells. All vials of McCoy cells were centrifuged, rinsed once with phosphate-buffered saline to reduce toxicity, refed with cycloheximide-minimum essential medium, then incubated for 48 hours. After incubation, one vial was fixed and then stained with fluorescein isothiocyanate-conjugated monoclonal antibodies. On all cultures that were initially negative, a subculture was performed using the duplicate vial. Coverslips were observed by fluorescence microscopy at a magnification of ×250. All positive cultures were assigned grades of 1+ to 4+ depending on the number of inclusion bodies seen by an experienced technologist who had no access to other test results. All coverslips showing signs of cytotoxicity were excluded from the study.

The collection supplies from TestPack Chlamydia and Surecell Chlamydia kits were used to obtain samples from subjects. All specimens were processed by a medical technician according to the manufacturer's specifications on the same day of collection. TestPack positivity was defined according to the manufacturer's instructions as a light to deep red hue, darker than the existing background, on the vertical line. Surecell positivity was defined as the appearance of a substantially darker red color

TABLE 1. COMPARISON OF TESTPACK AND SURECELL WITH CULTURE FOR ENDOCERVICAL *CHLAMYDIA TRACHOMATIS* DETECTION

	Test			
	TestPack*		Surecell†	
Test Performance	No.	(%)	No.	(%)
Sensitivity	31/60	(51.7)	46/60	(76.7)
Specificity	440/442	(99.5)	433/439	(98.6)
Positive predictive value	31/33	(93.9)	46/52	(88.5)
Negative predictive value	440/469	(93.8)	433/447	(96.9)

*TestPack Chlamydia, Abbott Laboratories, North Chicago, Illinois.
†Surecell Chlamydia, Eastman Kodak Co, Rochester, New York.

in sample well #2 compared with negative well #1. *C trachomatis* quality control testing was performed initially on each new test kit utilized.

Comparisons between the enzyme immunoassay test kit results on the same subjects were performed using McNemar's test with a Yates' continuity correction. The performance of each test was compared among different groups of subjects using Fisher's exact test or chi-square with Yates' correction for continuity. Logistic regression analysis[6] was used to evaluate *C trachomatis* risk factor variables.

RESULTS

During the study 512 patient specimens were tested for *C trachomatis,* and data are available for 502. Eight cell cultures that displayed cytotoxic effects and two samples lacking transport media were excluded from calculations. Three enzyme immunoassay samples were lost. There were 60 culture-positive patients identified. The study population prevalence of chlamydial infection was 12.0%. Two thirds (68%) of the patients were seen at the county health department and one third (32%) were seen at Planned Parenthood.

Patient demographics showed that 68% of subjects were black. The mean age was 24 years, with an average of one prior pregnancy. The method of contraception used by 43.3% of the women was oral contraceptive agents; barrier methods were used by 6.7%, and none or other methods were used by 50%. Approximately 82% of subjects claimed no prior history of a sexually transmitted disease, while a history of gonorrhea was reported by 9.9%. Most (82.5%) of the subjects screened were asymptomatic.

The results of the two enzyme immunoassay tests when compared with chlamydial culture are listed in Table 1. TestPack demonstrated a sensitivity of 51.7% compared with 76.7% for Surecell. The specificities were 99.5% and 98.6%, respectively. The positive and negative predictive values for TestPack were 93.9% and 93.8%, and for Surecell were 88.5% and 96.9%, respectively.

TABLE 2. POTENTIAL RISK FACTORS FOR ENDOCERVICAL *CHLAMYDIA TRACHOMATIS* INFECTION

Risk Factor	Odds Ratio (95% Confidence Interval)	χ^2	*P* value
Race, black	2.1 (0.9–4.5)	4.4	.11
Age, <25 years	2.6 (1.3–5.6)	6.5	.01
Gravidity	1.3 (0.6–2.7)	10.5	.23
Contraceptives, nonbarrier	0.7 (0.3–1.5)	6.6	.19
History of prior sexually transmitted disease	0.8 (0.4–1.7)	6.8	.45
Genital symptoms	0.7 (0.3–1.7)	1.2	.99
Cervical friability	1.6 (0.8–3.4)	17.7	.006
Uterine/adnexal tenderness	0.8 (0.2–3.0)	.01	.92

The greater sensitivity of the Surecell kit compared with the TestPack kit can be demonstrated by results from the 3+ to 4+ culture inclusion counts. Surecell had 6 false-negatives in the high (3+ to 4+, or >50 inclusion-forming units) inclusion count range, whereas TestPack had 19 false-negatives. Both kits, however, performed similarly in the lower inclusion count (1+ to 2+, or <50 inclusion forming units) culture range.

Potential risk factors for cervical *C trachomatis* are found in Table 2. Logistic regression analysis of patient demographics and historical and clinical findings showed that in this investigation only age younger than 25 years was a significant independent risk factor for *C trachomatis* cervicitis. Chi-square analysis also showed age younger than 25 years ($P = .011$) and cervical friability ($P = .006$) to be significant risk factors.

The institution of empiric treatment was compared with culture results and analyzed. The data indicate that 68% of culture-positive patients were not presumptively treated. Conversely, 18% of culture-negative patients were treated empirically with antibiotics.

The results of the operator performance in vitro investigation are found in Table 3. There were no differences in operator test performance among the four groups when compared for each test kit. There was variability between the two test kit results; however, this variability was independent of operators.

DISCUSSION

This investigation was designed to compare two rapid enzyme immunoassay test kits developed for the detection of endocervical *C trachomatis* infection. Unpub-

TABLE 3. COMPARISON OF OFFICE STAFF PERFORMANCE WITH *CHLAMYDIA TRACHOMATIS* ENZYME IMMUNOASSAY (EIA) TEST KIT

Group	Test							
	Testpack*				Surecell†			
	Sensitivity‡		Specificity§		Sensitivity		Specificity	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Physicians	6/6	(100)	18/18	(100)	5/6	(83)	15/18	(83)
Nurses	4/4	(100)	19/20	(95)	2/4	(50)	15/20	(75)
Medical technicians	4/4	(100)	20/20	(100)	3/4	(75)	20/20	(100)
Receptionist and x-ray technician	5/5	(100)	19/19	(100)	4/5	(80)	17/19	(89)
Total	19/19	(100)	76/76	(98.7)	14/19	(73.7)	67/77	(87.0)

**TestPack Chlamydia, Abbott Laboratories, North Chicago, Ill*
†Surecell Chlamydia, Eastman Kodak Co, Rochester, NY
‡Sensitivity—Positive EIA test result/positive diluted C trachomatis *sample.*
§Specificity—Negative EIA test result/negative control sample.

lished noncomparative independent investigations are small and few in number. Miller and Bovey[7] evaluated 91 patients with TestPack Chlamydia at a student health clinic with an infection prevalence of 15.4%. When compared with culture, TestPack had a 71.4% sensitivity, 91.1% specificity, 66.7% positive predictive value, and 94.7% negative predictive value. Reichart et al[8] studied 84 patients at a sexually transmitted disease clinic and found a 17.9% prevalence of infection. TestPack had a 67% sensitivity, 99% specificity, 91% positive predictive value, and 73% negative predictive value when compared with culture. In a multisite study of 1694 patients, Coleman et al[9] compared TestPack with culture. The overall infection prevalence was 11.6%. TestPack sensitivity when compared with culture was 72.9% and the specificity was 97.4%, with a 79.5% positive predictive value and 96.3% negative predictive value.

The present study had a different population base and swab collection sequence (with the first two swabs used for *N gonorrhoeae* and *C trachomatis* cultures), which may partially explain the lower sensitivity and predictive values for TestPack. A comparison of the test data indicates that TestPack outperformed Surecell in the in vitro testing. It is postulated, therefore, that the TestPack antigen extraction step may be less efficient than that of Surecell. A poor extraction procedure will result in less antigen release and consequently a decreased sensitivity. The superb culture method of the investigation laboratory, attributed to rapid same-day transport and testing performed by experienced personnel, substantiated by an 0.6% blind passage isolation rate, minimized reference test error. This fact may also influence the sensitivity when compared with other studies.

Chauncey et al[10] reported combined results from multicenter sites for 840 patients tested with Surecell Chlamydia. The average sensitivity was 85%, specificity 98%, positive predictive value 92%, and negative predictive value 96%. Some culture samples, however, were frozen or stored at 2°C to 8°C for up to 24 hours before cell inoculation; freezing decreases organism viability. Some sites sampled for direct fluorescent antibody tests and *N gonorrhoeae* culture after the EIA swab was used to collect the first specimen. The false-positive rate in the present study may be explained by wash technique error, filter failure, or cross-reactivity with other organisms. The sensitivity demonstrated for Surecell, although low, would make it the better choice to allow greater detection of disease.

The specificity for the TestPack was greater than for the Surecell, but may be clinically insignificant. False-positive test results may be due to culture insensitivity and error or to nonviable *C trachomatis* organisms.[11] The latter is a remote possibility, since samples were processed the day of collection, and potential subjects who had received antibiotics within the preceding month were excluded. Viable *C trachomatis* organisms may be detected by cervical culture up to 5 days after initiation of antibiotic therapy. An enzyme immunoassay test has been shown to detect nonviable organisms for up to 10 days following initiation of antibiotic treatment.[12] There exists a 5-day time lag for cervical chlamydial antigen clearance.

The 12% prevalence of infection in this study population is considered moderate for *C trachomatis* cervicitis. Higher or lower disease prevalences will influence the test predictive value results. For example, a commonly reported prevalence for *C trachomatis* cervicitis in a family practice setting is 5% to 12%.[13,14] The positive predictive values of EIA tests would be lower in settings with a lower disease prevalence.

To determine the validity of the comparative sensitivity results, in vitro serial dilution testing was performed. A stock solution of a single strain of *C trachomatis* was

serially diluted. Both enzyme immunoassay tests were evaluated at each dilution. Surecell demonstrated the ability to detect a one-half log greater dilution than TestPack. This finding may support the observed clinical investigative difference between the EIA test kit sensitivities.

The results from the operator performance variability testing indicate that those office staff positions studied are capable, after routine instruction, to perform the enzyme immunoassay tests reliably. Personnel expertise in this case does not appear to be of major importance in test performance. The greater sensitivity of TestPack demonstrated in this in vitro investigation resulted from a lack of required *C trachomatis* extraction from cells and a better test result indicator for observers to interpret low-level positive results.

Both kits are easy to use and relatively inexpensive ($8 to $10 per test), but require refrigeration of some reagents. To increase productivity and efficiency in a busy office, four to six tests may be processed simultaneously by each kit. The TestPack requires smaller refrigerator storage space. The filter device and ease of test interpretation are notable. A vortexer is required, however, and is not included with the kit. The Surecell extraction block and filter membrane evaluated in this study were considered to be weaknesses. Since this investigation, both tests kits have been modified and improved. Both kits easily allow for quality control testing, which is necessary for the office laboratory to meet the established federal guidelines.[15]

Diagnostic testing for *C trachomatis* is clinically important, since a majority of infected women are asymptomatic, and clinical findings suggestive of *C trachomatis* infection are unreliable and nonspecific. As demonstrated in this investigation, 68% of culture-positive patients would not have been treated without testing.

Given the reported sensitivities of these test kits, their ability to perform well in population prevalences of 5% or less is questioned. It is suggested that, as was done in this study, selective screening using previously identified positive risk factor predictors for *C trachomatis* cervical infections[5] be followed when utilizing these kits. By thus increasing the population prevalence, the predictive value of the test may be improved.

Further enzyme immunoassay test improvements are necessary, as there is a critical need for these tests in office laboratories. It is hoped that newer products with improved sensitivity will promote expanded screening and assist in the epidemiologic control of genital *C trachomatis* infections.

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